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THE NERVOUS COMPONENT OF THE ATRIOVENTRICULAR BUNDLE

By E. J. FIELD*

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In his monograph *Das Reizleitungssystem des Säugetierherzens* Tawara (1906) described numerous nerve fibres accompanying the atrioventricular bundle and its branches. He spoke of 'außerordentlich zahlreichen Nervenbündel' in the left branch of the bundle (calf) some of which when traced distally '...den linken Schenkel verlassen und sind entweder subendokardial oder intermuskular nach anderen Richtungen gezogen' (p. 107 loc. cit.). It would appear from this description and from the beautiful illustrations given by Wolhynski (1928) that a fully fledged alternative conducting system between atria and ventricles exists. Wilson (1909), Engel (1910), Meiklejohn (1914) and Scaglia (1927) all found nerves within the bundle but considerable species differences were reported. Woollard (1926) claimed that very fine fibres of the intracardiac network actually penetrated the substance of individual heart-muscle fibres and ran therein for considerable distances giving off at intervals small terminal loops and swellings. Much the same appearances were recorded, too, by Boeke (1932). These anatomical findings have, however, received scant recognition in current views of the mechanism of the heart beat, though strong criticism has come recently from Glomset and his co-workers (1940, 45, 48). In view of the importance of anatomical structure as a sound basis for physiological theory, it was decided to examine the nervous component of the atrioventricular bundle in a variety of animals.

MATERIAL AND METHODS

The animals examined were sheep (foetal and adult), rabbit, guinea-pig, rat, cat, dog, macaque, chimpanzee, gorilla and man. From each heart a block of tissue was cut comprising the interatrial septum and the upper part of the interventricular septum. Fixation was in 10% neutral formalin and frozen sections at 40μ were impregnated by the method of Bielschowsky-Gros. In the case of the gorilla heart and in one human heart paraffin sections (10μ) were prepared and stained by Bodian's method. Methylene blue was tried initially with rabbit material, and although some satisfactory preparations were made they did not compare in clarity with the silver method. Difficulties of interpretation as between nerve fibres and connective tissue strands have been remarkably few with the silver method—fewer than with methylene blue. One or two technical points may be mentioned. The initial silver bath (20% silver nitrate) should not be prolonged beyond 1 hr., 30–35 min. is quite satisfactory. In making up the ammoniacal silver solution, whilst no definite rule can be given as to the quantity of ammonia which should be incorporated, it does seem important to add the ammonia a few drops at a time and

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to shake well immediately after each addition. It is not satisfactory to add in one stage the approximate amount of ammonia required and then finish off by adding drops.

OBSERVATIONS

Sheep. Numerous bundles of nerve fibres are embedded in the substance of the atrioventricular bundle. Many appear to originate in scattered masses of ganglion cells in the lower part of the interatrial septum in and around the atrioventricular node. At the bifurcation of the bundle nerves pass into both right and left branches (Pl. 1, fig. 1). These nerves may leave a branch and pass for considerable distances subendocardially (Pl. 1, fig. 2) before turning into the myocardium. The further fate of these nerves is difficult to follow. Nerve fibres also accompany the larger blood vessels, though endings within the media were never seen. It is interesting to recall that Stöhr (1928) never found nerve endings stainable with silver within the media of vessels, though Blair & Davies (1935) claim to have demonstrated them. Within the myocardium there is a beautiful network of capillaries elongated in the direction of the heart muscle fibres and so rich that fibres and capillaries alternate. In more or less close relation to these capillaries fine non-myelinated nerve fibres twist and turn but never, apparently, form nerve endings on capillary walls. The appearances suggest a closed network of nerve fibres as described by Stöhr (1928). Many of the fine nerve fibres pass very close to muscle fibres and some even seem to pass through their substance, though it is surprisingly difficult to be certain of the relation of nerve fibres to muscle cells even at high magnification with stereoscopic eyepieces. Certainly no nerve terminations of the type depicted by Boeke (1932) were found, though admittedly he employed a slightly different staining technique. It seems probable that there is continuity between the nerve fibres leaving the bundle branches and the intramural network, for the origin of which it would be difficult to account; but in the present material this continuity could not be established with any degree of certainty. An origin from the nerve plexus accompanying the coronary vessels is conceivable, for there is undoubtedly a rich plexus surrounding the larger vessels. In spite of numerous attempts, however, it has not been found possible to demonstrate more than a few nerve fibres in association with the smaller coronary vessels, and certainly not sufficient to account for the rich plexus within the myocardium.

Rabbit. Numerous non-myelinated fine nerve fibres pass amongst the cells of the atrioventricular node, coming into intimate contact with muscle cells (Pl. 1, fig. 3). The bundle and its branches are poorly developed and the latter can be followed for only a short distance on each side of the upper part of the interventricular septum. The nerve fibres of the node do not become massed into definite strands within the bundle, but individual fibres interlace with muscle cells. Moreover, many nerve fibres surround the bundle and are not actually within its substance. Once the bundle reaches the upper part of the interventricular septum considerable numbers of nerve fibres pass off into the septum. Biggs (1908), working with the isolated perfused rabbit heart, found it impossible to produce marked or permanent dissociation between atria and ventricles by extensive lesions of right and left bundle branches in the interventricular septum. He came to the conclusion that there

must be some other path of conduction which could only be divided by an incision at, or before, the bifurcation of the atrioventricular bundle. It has been claimed that such results may be due to the existence of aberrant conducting fibres which leave the main atrioventricular bundle to pass independently through the fibrous skeleton of the heart. In two rabbit hearts examination of complete serial sections of the bundle and its division failed to show any such aberrant fibres. The arrangement of the nervous component of the bundle as described above is such that only wide incisions in the upper part of the interventricular system are likely to divide it completely. If the nerves of the bundle do indeed play an important part in conduction of the heart beat, then their arrangement might well explain the extensive nature of the lesions which Biggs found necessary to produce dissociation. This finding has recently been confirmed in unpublished work by Field & A. F. Rogers.

Rat. The bundle and its branches are poorly defined histologically in this animal. Numerous fine non-myelinated fibres pass through the atria to the ventricles in the neighbourhood of the bundle rather than within its substance (Pl. 1, fig. 4). Many show well-marked beading (Pl. 1, fig. 5). Having reached the interventricular septum the nerves quickly become lost within it.

Guinea-pig. In this animal the nervous component of the bundle is more compact than in the rabbit or rat, its arrangement resembling rather that obtaining in the sheep. The bundle itself is somewhat more easily made out than in the rat, and once again the nerve fibres within it pass off into the upper septal musculature (Pl. 2, fig. 6).

Dog. The atrioventricular node in this animal is permeated through and through with a remarkable network of fine non-myelinated nerve fibres (Pl. 2, fig. 7). The photograph hardly gives an adequate impression of the richness of fibres within the node. As examination is made under high magnification with the fine adjustment of the microscope more and more nerve fibres come into view intimately related to muscle cells. Nerve endings are, however, conspicuously absent. Within and around the bundle are more or less compact nerve fascicles; occasional fibres appear to have a myelin sheath (Pl. 2, fig. 8). In the upper part of the interventricular septum many nerve bundles forsake the conducting system and pass into the myocardium. At intervals individual fibres separate from these bundles. The richness of the nervous component of the conducting system in the dog is striking, and is of especial interest as this animal has been so widely used experimentally in work on the conduction of the cardiac impulse.

In the lowest part of the interatrial septum, in and around the region of the atrioventricular node, there are many islands of ganglion cells from which the nerves of the bundle take origin (Pl. 2, fig. 9).

Macaque and Chimpanzee. Ganglion cells are frequently met with in clusters in the lower part of the interatrial septum of these animals, and in the macaque they are occasionally binucleated and lobulated. Within the conducting system of these animals—not at all well developed histologically—there are relatively few nerve fibres as compared with the previous animals described, and the few there are appear as isolated fibres (Pl. 2, fig. 10). On two occasions, in some fifty sections, the writer has come across what appear to be nerve endings in relation to muscle cells in the bundle of the macaque. These endings are very minute but they do bear some resemblance to those described in the general myocardium of the sheep

(Boeke, 1932). If these be true nerve endings they must be very rare or very difficult of impregnation. They appear to be of digitate type and on focusing up and down a suggestion of a periterminal network may be made out.

In the subendocardium of the interventricular septum fine beaded nerve fibres were found in the chimpanzee (Pl. 3, fig. 11). Many end in small terminal enlargements immediately beneath the endocardium. It was not possible in the sections prepared to determine whether these fibres were in continuity with those of the atrioventricular bundle. The intramural nerve plexus described above in the sheep heart was particularly well impregnated in the chimpanzee myocardium and appeared to be of the same character (Pl. 3, fig. 12).

Gorilla. The atrioventricular bundle in this animal contains no discrete nerve fascicles. The bundle was sectioned transversely—an unfavourable plane for picking out longitudinally running nerve fibres, but there do seem to be some fine non-myelinated, beaded fibres associated with it, especially near its periphery (Pl. 3, fig. 13). These fibres are gathered together into more compact bundles on reaching the interventricular septum and can be found coursing within the myocardium (Pl. 3, fig. 14). Their mode of termination could not be discovered.

Man. In relation to the atrioventricular node and bundle there are considerable numbers of fine nerve fibres, more especially around the bundle than in it (Pl. 3, fig. 15). Some of the nerve fibres leave the vicinity of the bundle to pass through the fibrous tissue surmounting the interventricular septum.

DISCUSSION

In or associated closely with all the atrioventricular bundles examined considerable numbers of nerve fibres were found. These fibres in some animals (sheep) are gathered together into discrete fascicles whilst in others with less well developed bundles they tend to run singly in a tortuous fashion. It would seem that when the bundle is well developed it acts as a great high-road from atria to ventricles and many nerve fibres pass within it. It may be that the general looseness of texture of the bundle and the fact that it develops in a region of relative quiescence (Field, 1950) make it a particularly favourable stroma for nerve fibres. When the nerve fibres are less numerous they tend to travel singly and often pass in the general neighbourhood of the bundle rather than within its confines. Lawrentjew & Gurwitsch-Lasowskaja (1930) studied the nerves of the atrioventricular bundle in the rat. They were struck by the 'erstaunliche Reichtum des Atrioventrikularbündels an Nervenelementen' (p. 591), and described the nerve fibres as forming a superficial plexus on the surface of the bundle and a deeper one within it. This latter, they claimed, consisted of fine non-myelinated fibres, whilst the former contained some myelinated fibres also. They found small ganglion cells (6–10 μ) scattered in the superficial plexus and noted their poorly argentophil character, it being necessary to overstain the preparation in order to render them at all visible. Deliberate overstaining brings in difficulties of interpretation because of connective tissue impregnation, and all preparations in the present work which showed connective tissue staining have been rejected. Lawrentjew & Gurwitsch-Lasowskaja were never able to see fibres leaving the neighbourhood of the bundle to pass off into the general myocardium. The present

material, however, affords many instances of such an occurrence both in lower animals (Pl. 2, fig. 6) and in man.

It must be recognized, therefore, that a nervous atrioventricular pathway exists in and around the atrioventricular bundle. Moreover, this nervous pathway is so intimately related to the bundle that lesions, pathological (e.g. Perry & Rogers, (1934), or experimental, must necessarily involve both the muscular and the nervous component. This fact, of outstanding importance, was clearly recognized by Cohn & Trendelenburg as long ago as 1910. They wrote: 'Man wird also immer im Auge behalten müssen, daß selbst bei einem Schnitte, der auf das genaueste die Grenzen der muskulären Bündelfaserung einhält, eine Unzahl feinster Nervenfasern durchschnitten wird so daß eine funktionelle Ausschaltung lediglich der Muskelfasern des Bündels ein Ding der Unmöglichkeit ist' (p. 84). From their careful and detailed study of the effects of lesions produced in the course of the atrioventricular bundle these authors cautiously drew the conclusion that 'Es kann nunmehr mit Sicherheit gesagt werden, daß dieser Weg auf das Engste mit dem Übergangbündel (worunter in diesem allgemeinen Sinne stets Muskulatur mit Nervengespinnst verstanden ist) zusammenfällt' (p. 84). Nevertheless, since that time attention—particularly from the clinical standpoint—has been fixed almost exclusively on the muscular component. It is difficult to establish the origins of such an unbalanced outlook, particularly as Wilson (1909) was driven to the conclusion that 'The neurologist might well refuse to recognize in...(the atrioventricular bundle)...a muscle bundle; to him it might become conspicuously a nerve pathway of very intricate structure' (p. 157). It may be that emphasis has fallen mainly on the muscle of the bundle because of the relative ease with which it can be examined histologically as opposed to the more complicated methods, often difficult of interpretation, necessary for the demonstration of the nervous component.

Some authors have described ganglion cells in the atrioventricular bundle at its bifurcation and in the proximal parts of the bundle branches, Tawara (1906), Lawrentjew & Gurwitsch-Lasowskaja (1930). The latter authors draw attention to the difficulty of demonstrating such cells, for even when the nerve fibres are adequately impregnated they appear very pale. In the present material nerve cells could not be identified with certainty within the atrioventricular bundle or its branches, though they were numerous in the region of the atrioventricular node. Engel (1910), using a methylene-blue technique on the calf heart, was unable to demonstrate the rich pericellular plexus described by Wilson (1909) within the bundle, but claimed that its innervation did not differ materially from that of the general myocardium. For him the thing which distinguished the bundle was the presence of the large nerve trunks which passed within it and divided to pass into its right and left divisions. Engel estimated that in the calf bundle there were about twice as many non-myelinated as myelinated fibres. Fukutake (1925), again using a methylene-blue method, described a fine nerve plexus in association with the fibres of the general myocardium and encompassing also the Purkinje system. From his description and illustrations, however, it is probable that he was dealing, to some extent at all events, with connective tissue fibres. Vitali (1937) has described a very rich network of delicate nerve fibres surrounding each Purkinje cell and penetrating within the cytoplasm to end in the neighbourhood of the nuclei. He is inclined to regard the

pericellular network as sensory in function, and is of the opinion that in its varying stimulation during different phases of the cardiac cycle it may aid in the regulation of the heart beat. Nonidez (1943), however, is of the opinion that the fibres described by Vitali are part of the argyrophil reticulum present everywhere in the myocardium. Scaglia (1927) found such large numbers of nerve fibres within the atrioventricular bundle that he came to the conclusion that they were not expended solely in the innervation of Purkinje tissue, but passed also to other parts of the heart. Mingled with these nerve fibres Scaglia found nerve cells either singly or in small groups. These findings were limited to the heart of *Bos taurus*, but the author expressed the opinion that fundamental species differences were unlikely.

FUNCTION OF THE NERVOUS COMPONENT OF THE ATRIOVENTRICULAR BUNDLE

It is generally accepted that the vagus nerves depress the activity of the conducting system, though the mechanism of such action remains obscure. The nerve fibres within the atrioventricular bundle are regarded for the most part as postganglionic autonomic fibres regulating conduction of the cardiac impulse carried by the specialized muscle of the bundle. Whilst it is not proposed to re-open here the question of myogenic as against neurogenic conduction of the heart beat, it should be emphasized that there exists between atria and ventricles a considerable nervous pathway, intimately related to the muscular bridge so widely accepted as the true conductor of the cardiac impulse. It must further be noted that the relation between the nervous and muscular components of the bundle is so close that it is impossible by experimental means to destroy one without the other (Cohn & Trendelenburg, 1910). The numerous experiments of this type only show that the path of atrioventricular conduction resides in the bundle, but cannot testify as to the relative parts played by the muscle and nerve components. Some evidence in this respect is, however, afforded by the work of Agduhr (1933) and Agduhr & Stenström (1928). They showed that cod-liver oil in certain dosages produced focal areas of degeneration in the myocardium. In the course of large numbers of experiments on mice it might occasionally happen that such lesions involved the atrioventricular bundle without necessarily producing a complete heart block. In one such heart Agduhr (1933) was able to find seemingly intact nerve fibres in the vicinity of the atrioventricular bundle. On the basis of his observations he raised the question whether it might not be the nerve fibres rather than the specific muscle tissue which were the conducting elements in the bundle. Wahlin (1935) was able to confirm Agduhr's observations. He found that sclerotic patches developing in the course of the atrioventricular bundle might be associated with a normal prolonged P-R interval but did not necessarily entail atrioventricular dissociation. Such cases showed intact nerve fibres passing from atria to ventricles in the vicinity of the bundle. These observations he regarded as 'eine Stütze fur die neurogene Auffassung daß die Nerven und nicht die muskuläre Komponente des His'schen Bündels das Substrat der Reizleitung zwischen Vorhof und Kammer darstellen' (p. 86).

It is interesting to find that Glomset and his co-workers (1940–8), from their extensive review of the literature and examination of clinico-pathological material,

are also inclined to afford the intrinsic nervous system of the heart an important role in cardiac action. Indeed they reach the conclusion that 'an atrioventricular muscular conduction system does not exist in any of the species studied. Therefore, the present concept of cardiac conduction is as inaccurate as was the concept of the circulation in preharveian days' (1948, p. 170).

SUMMARY

The nervous component of the atrioventricular bundle has been described in sheep, rabbit, guinea-pig, rat, cat, dog, macaque, chimpanzee, gorilla and man. The possible significance of these fibres is briefly discussed.

It is a pleasure to record my indebtedness to Prof. J. M. Yoffey for his continued interest and encouragement, and to the late Prof. Sir Joseph Barcroft for the foetal sheep material. I should like to thank Messrs J. E. Hancock and L. Cooper for help with the photographs.

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EXPLANATION OF PLATES

Plate 1

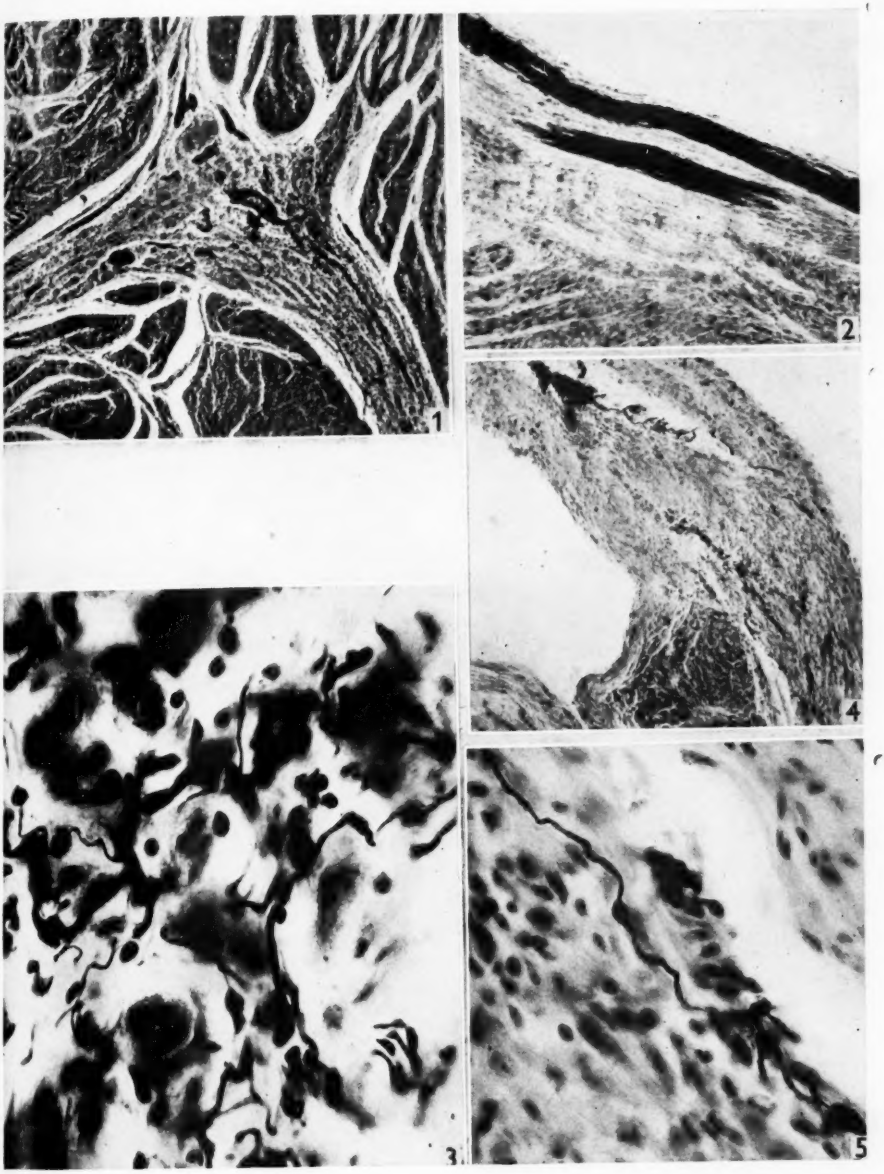
Fig. 1. Coronal section through the upper interventricular septum of a 38 cm. sheep embryo. The bifurcation of the atrioventricular bundle is seen straddling the septum and bundles of nerve fibres are present within the right and left branches. × 110.
Fig. 2. Right face of interventricular septum showing subendocardial nerve bundles derived from the right branch of the His bundle. 38 cm. sheep embryo. × 110.
Fig. 3. Atrioventricular node of rabbit heart. Numerous fine beaded nerve fibres are seen intertwining with pale muscle cells. × 780.
Fig. 4. Coronal section through rat heart. Numerous nerve fibres are seen in association with the atrioventricular bundle. × 110.
Fig. 5. High power view of fig. 4. Beaded character of the atrioventricular nerve fibres is apparent. × 850.

Plate 2

Fig. 6. Coronal section through upper part of interventricular septum of guinea-pig. To the right is the left branch of the atrioventricular bundle accompanied by a nerve bundle. A single nerve fibre is seen leaving this bundle and passing off with the septal musculature. × 350.
Fig. 7. Atrioventricular node of dog heart. A very rich plexus of fine nerve fibres is seen intermingled with the tangled and rather poorly staining muscle fibres of the node. × 700.
Fig. 8. Small bundle of nerve fibres accompanying the atrioventricular bundle of the dog. One fibre shows a distinct myelin sheath.
Fig. 9. Group of ganglion cells in the lowest part of the interatrial septum; giving origin to some of the nerve fibres of the His bundle. Dog heart. × 400.
Fig. 10. Macaque atrioventricular bundle. Single nerve fibre coursing between poorly differentiated muscle cells. × 1000.

Plate 3

Fig. 11. Chimpanzee heart. Right face of interventricular septum showing fine beaded subendocardial nerve fibres some of which seem to end in terminal knobs beneath the endocardium itself. × 650.
Fig. 12. Chimpanzee: interventricular septum. Fine beaded nerve fibres accompanying capillaries between muscle fibres. These nerve fibres seem to be organized into a closed network. × 650.
Fig. 13. Gorilla. Transverse section through atrioventricular bundle showing fine beaded nerve fibres round its periphery. × 450.
Fig. 14. Gorilla. Interventricular septum. Strand of nerve fibres which has come through with the bundle of His ramifying in the muscle of the septum. Bodian stain. × 100.
Fig. 15. Human. Left branch of His bundle, accompanied by strand of nerve fibres. × 400.



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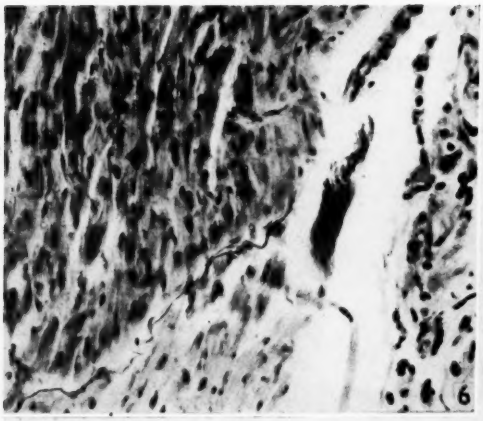
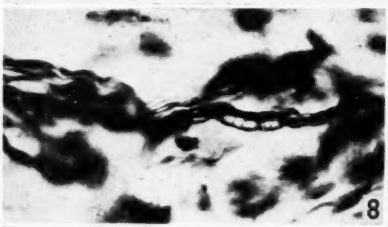
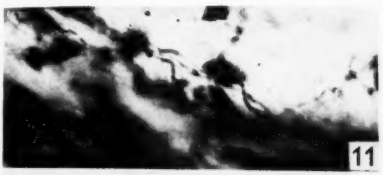
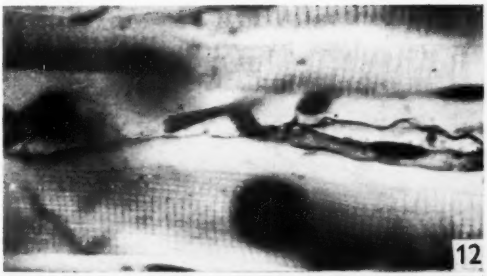
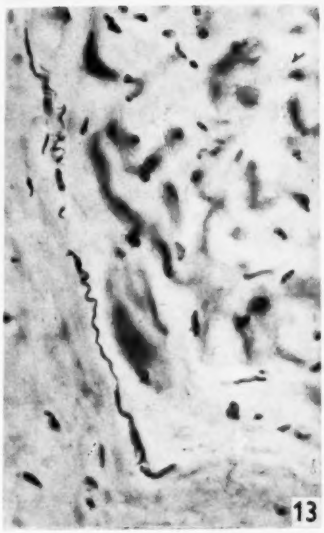


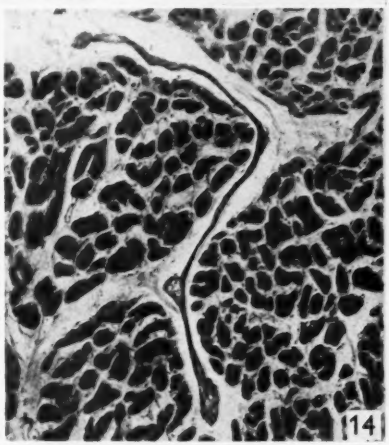


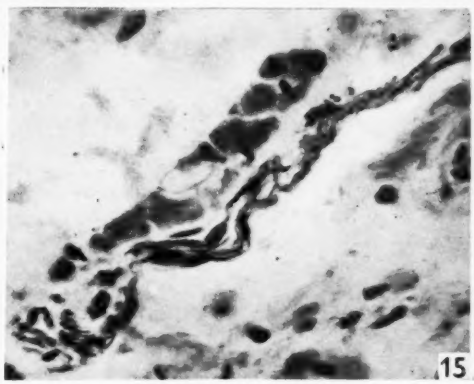
FIELD—THE NERVOUS COMPONENT OF THE ATRIOVENTRICULAR BUNDLE











FIELD—The nervous component of the atrioventricular bundle

THE STRUCTURE AND FUNCTION OF THE CHOROIDAL GLAND OF TELEOSTEAN FISH

By C. H. BARNETT
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INTRODUCTION

Albers (1806) was the first to show that the horseshoe-shaped body found in the choroidal layer of the eye in most bony fish is neither a muscle nor a secretory gland, as was currently supposed, but a collection of small blood vessels—a form of rete mirabile. In 1838 W. Jones published a much more detailed description, having attempted with little success to inject these vessels with mercury. Müller (1839), in his classical study upon the eyes of fishes, pointed out that all the blood to this choroidal gland had first to pass through the pseudobranch. Allen (1905) successfully injected the choroidal vessels, and later (1949) gave a detailed account of them as seen by dissection. Allis (1912) emphasized that the ophthalmic (or efferent pseudobranchial) artery had no communication with neighbouring vessels.

In this investigation an attempt has been made to ascertain the arrangement of blood vessels in the choroidal gland and in the lentiform body, a small oval vascular body also lying in the choroid layer, and to interpret this arrangement in terms of function.

MATERIAL

Several species of fish have been examined, in most cases by study of serial sections as well as by dissection; they include the cod (*Gadus morrhua*), haddock (*G. aeglefinus*), pike (*Esox lucius*), roach (*Rutilus rutilus*), carp (*Cyprinus carpio*), salmon (*Salmo salar*), rainbow trout (*S. irideus*), eel (*Anguilla anguilla*) and catfish (*Amiurus*).

The rainbow trout chiefly has been studied, for the following reasons:

(*a*) It has a large eye and reputedly good vision.

(*b*) Its eye shows a typical teleostean structure, including lentiform body, falciform process and retractor lentis muscle.

(*c*) It is easily kept in a laboratory tank, and all ages are readily available.

METHODS USED IN STUDYING THE RAINBOW TROUT

(*a*) Dissection of the eyeball, bisected after freezing in a salt-ice mixture.

(*b*) Examination of serial sections of whole eyeballs and isolated choroidal glands, in paraffin and celloidin.

(*c*) Injection through the dorsal aorta of indian ink in plasma (Holmes & Davies, 1948) followed by sectioning as above.

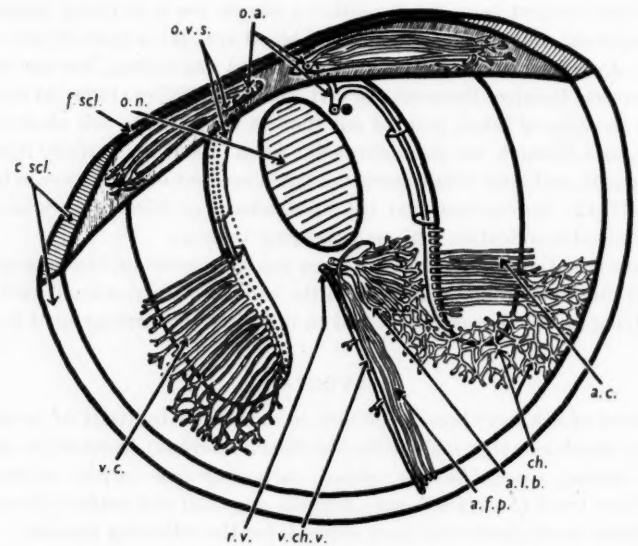
(*d*) Injection of Neoprene latex, using the technique of Trueta, Barclay, Daniel, Franklin & Prichard (1947), followed by dissection, with and without maceration.

(*e*) Sectioning whole heads of young trout.

ANATOMY OF THE CHOROIDAL VESSELS

Immediately adjacent to the retina there is an extensive capillary network, the choriocapillaris layer, which is fed by two arteries—the wide thick-walled *ophthalmic* artery, and the narrower *retinal* artery which supplies also the falciform process and the retractor lentis muscle.

The *ophthalmic* artery springs from the pseudobranch and passes direct to the eyeball. It pierces the sclera behind and dorsal to the optic nerve and passes into the lumen of the wide ophthalmic venous sinus that lies along the inner border of the choroidal gland. Therein it divides into two branches, which run along the inner border of each limb of the horseshoe-shaped gland, giving off parallel twigs into it,



Text-fig. 1. The vascular pattern of the choroidal gland and lentiform body (rainbow trout, right eye). The upper part of the eyeball has been cut away to show the limbs of the choroidal gland in cross-section.

so that clusters of large capillaries arise almost directly from the thick-walled artery (Pl. 1, fig. 1). These capillaries radiate in almost parallel columns, occasionally giving off collateral branches, towards the periphery of the gland, from which they emerge and pass into the choriocapillaris network (Pl. 1, fig. 2). Many turn towards the centre of the retina, running between the choroidal gland and the basal retinal cells, and a free anastomosis of large capillaries occurs around the optic nerve.

Blood is returned from the choriocapillaris vessels by venous capillaries which enter the periphery of the gland and pass centrally through it to open into the *ophthalmic venous sinus*; this drains into the large ophthalmic vein. A constant feature, revealed by injection, is that each venous capillary is sandwiched between

arterial capillaries. The two streams are separated only by single-layered endothelial walls (Pl. 1, figs. 5, 6).

The *retinal* artery, from the internal carotid, supplies the optic nerve (by branches that enter it outside the eye and divide in a T-shaped manner) and the extra-ocular muscles and fat (by means of a free network of large vessels). It then penetrates the membranous portion of the sclera, along the ventral surface of the optic nerve, and at once enters the *lentiform body*. It lies anterior to the root of the falciform process, which projects as a low ridge between the limbs of the choroidal gland, pointing forwards and ventrally. Within the lentiform body, the artery lies inside a small venous sinus and sends parallel, but somewhat tortuous, branches through this body to emerge as the capillaries that constitute the central part of the chorio-capillaris layer. These freely communicate with the capillaries fed (via the choroidal gland) by the ophthalmic artery.

One slightly larger vessel, usually at the dorsal margin of the lentiform body, does not enter the choriocapillaris, however, but loops first dorsally then forwards and ventrally, often crossing superficial to the retinal artery, to enter the root of the falciform process. It runs deep to this, dividing as a rule into three or four finer vessels, as far as the retractor lentis muscle, which it helps to supply with blood. It is accompanied by the motor nerve to this muscle.

The venous drainage of the falciform process is via a large *ventral choroidal* vein. This vessel also receives a few capillaries from the choriocapillaris layer. It is joined by a large vein from the lentiform body to form the *retinal* vein, which pierces the sclera beside the corresponding artery. The vein from the lentiform body is made up by the union of venous capillaries that arise from the choriocapillaris plexus; these alternate within the lentiform body with the arterial capillaries.

At its origin the ventral choroidal vein shows some capillary connexions with the *iris* artery given off from the external carotid. This artery does not supply the choroidal gland and need not be described in detail—its course in the trout is similar to that in the Loricati (Allen, 1905).

Thus the blood vessels of the eye of the trout may be divided into two almost entirely independent groups:

(i) Those vessels supplying the choriocapillaris plexus via the choroidal gland or the lentiform body. The striking feature of this group of vessels is the regular alternation of arterial and venous capillary streams.

(ii) Those vessels supplying the ciliary region, the optic nerve, and the extra-ocular tissues. The artery to the falciform process may be included here—it passes along the dorsal edge of the lentiform body but, unlike the first group of vessels, does not run between venous streams.

Allen's statement (1905) that the ophthalmic artery in the Loricati has no connexion with other arteries is equally true for the trout. This has been confirmed by examining serial sections of the decalcified heads of young fish. Moreover, in adult fish, Neoprene injections will sometimes fill the internal carotid artery but not the ophthalmic (since before filling the latter the injection mass has to flow through the capillaries of the pseudo-branch). Maceration of such an eye shows a well-filled lentiform body (Pl. 1, fig. 7), but no Neoprene at all within the choroidal gland.

FUNCTIONS OF THE CHOROIDAL GLAND AND LENTIFORM BODY

Several hypotheses have been considered, and attempts have been made to confirm or disprove them. Certain views, e.g. that the organs are actively secretory, may be ruled out as based upon incorrect anatomy. Four possibilities merit detailed consideration:

(*a*) *The choroidal gland as an erectile organ*

According to this view, the gland is a mass of erectile tissue, expanding to push forward the retina and thus acting as a focusing mechanism (Rymer Jones, 1855).

Differential staining of the walls of the ophthalmic artery reveals that they are mainly fibrous, with only a few plain muscle fibres. The possibility remains that the pseudobranch is the 'tap' that restricts or increases the blood flow to the choroidal gland.

To verify whether the gland could increase in thickness, the following experiment was performed:

Amyl nitrite, 0·5 c.c./2 l., was added to the water in which a fish was kept. Five minutes later, the fish was plunged into a freezing mixture of carbon dioxide and alcohol. This killed the fish instantaneously, and the dead fish was then placed in Bouin's fluid while it slowly thawed. The blood vessels were thus fixed in their dilated state. Forty-eight hours later, the eyeball was removed and serial sections cut. It was found that the choroidal gland, though packed with red cells, was no thicker than in a similar fish treated with ergometrine tartrate instead of amyl nitrite. The difference caused by the drugs in the quantity of blood in the ophthalmic artery and vein and in the choriocapillaris layer was striking (Pl. 1, figs. 3, 4). Adrenaline hydrochloride and Pitressin likewise caused no flattening of the choroidal gland.

Repeated experiments with pairs of trout of varying ages from 3 months to 2 years, one fish of each pair being used as a control, confirmed the lack of effect on the thickness of the choroidal gland of vasodilator or vasoconstrictor drugs.

As further proof in the living fish, the retinae of large trout were examined by means of an ophthalmoscope and retinoscope through the wall of a narrow Perspex tank in which the fish had been placed after narcotizing with Nembutal. Amyl nitrite did not make the eye more hypermetropic, even after its retractor lentis muscle had been paralysed by atropine sulphate (Beer, 1894).

These experiments seem to rule out the possibility that the choroidal gland is an actively erectile organ.

(*b*) *The choroidal gland as a mechanism to reduce pulsation of the retina*

This is a popular theory (Franz, 1934; Walls, 1942). The eyes of fish have such a short focal length that slight pulsations of arteries deep to the retina would impart movements to it great enough to disturb the accurate focusing of an image. By passing the arterial blood through a rete of capillaries, pressure waves would be 'ironed out'. However, the blood to the choroidal gland has already passed through two sets of capillaries, namely those of the gills and the pseudobranch, and persistence of any pulsation would be very unlikely.

To confirm this supposition, trout embryos within a week or two of hatching were examined alive under a binocular microscope. Through the transparent body of the embryo the ventral aorta could be seen pulsating but, unless the embryo was dying, very little pulsation was seen in the blood leaving the gills.

One of the functions of the pseudobranch in the adult may be to eliminate the last traces of pulsation in the blood reaching the eye.

(*c*) *The choroidal gland as a passive reservoir*

Rochon-Duvigneaud (1943) has stated that the choroidal gland, together with the intraocular serous sacs, acts as a 'bellows', flattening by expulsion of blood when the intraocular pressure is raised, as in diving to deeper levels. Allen (1949) favours a somewhat similar view.

It is, however, very difficult to reduce the thickness of the choroidal gland by squeezing it between flat plates. Moreover, its existence in almost all bony fish, irrespective of the depth at which they live and feed, makes this view unlikely.

(*d*) *The choroidal gland as an organ of biochemical interchange*

The best explanation of the remarkably regular pattern of alternating arterial and venous streams in both the choroidal gland and the lentiform body is that these organs are comparable in function to a placenta, allowing a certain amount of diffusion across the capillary endothelium.

It is possible that the retinal cells of these fish contain a substance, essential for vision, of such a low molecular size that it tends to diffuse out into the choriocapillaris vessels. To prevent its loss into the general blood stream it is at once shunted back through the choroidal gland into the arterial capillaries.

Supporting this idea is the fact that the choroidal gland is absent in certain small-eyed fish (e.g. catfish, eel) which are usually inhabitants of muddy water or are night feeders; in other words, they have eyes that do not require (or are unable) to penetrate a long distance through water.

The strict relationship of the pseudobranch to the choroidal gland is not easily explained. In some fish, the former is undoubtedly capable of adding more oxygen to the already oxygenated blood it receives from the gills (Granel, 1923) and may therefore compensate for what is perhaps an inevitable loss of some oxygen across the endothelial walls of the choroidal gland vessels. In other fish, however, it is buried too deeply to be directly respiratory in function, although it would still be capable of altering the pH, osmotic pressure, etc., of the blood passing through it, so that diffusion of respiratory gases within the choroidal gland might be minimized.

In order to test whether there is any substance within the vessels of the choroidal gland that is not present in the general circulation, Dr H. J. A. Dartnall very kindly undertook to measure the absorption spectra of 2% Digitonin extracts of blood taken from the choroidal gland, pseudobranch and caudal artery.

Whereas the blood in the pseudobranch and the caudal artery showed absorption bands of haemoglobin only, that taken from the choroidal gland of both the rainbow trout and the roach showed in addition an intense band at about 4070 Å. and a weaker band extending between 4700 and 5500 Å.

This spectrum resembles that of oxidized cytochrome *c*, a substance which may be associated with oxidative mechanisms in the eye (Dartnall & Thomson, 1949). It is hoped to carry out further experiments to demonstrate whether the substance present in the choroidal gland is actually cytochrome *c*.

SUMMARY

The arrangement of blood vessels within the choroidal gland and lentiform body of the teleostean eye has been studied by injection techniques. The capillaries passing through these organs to enter the choriocapillaris plexus regularly alternate with the venous capillaries leaving it.

Numerous experiments have been carried out to ascertain the function of these vascular bodies. It seems most likely that they are organs of biochemical interchange, preventing the loss into the general circulation of some substance which diffuses out of the retinal cells into the choriocapillaris vessels. The absorption spectrum of blood extracted from the choroidal gland indicates that this postulated substance may be cytochrome *c*.

I wish to thank Prof. D. V. Davies for his constant guidance and encouragement. My thanks are also due to Prof. H. A. Hartridge, Mr. J. S. Phillpotts and Dr W. Rushton for help and advice on different aspects of this subject. Mr E. King gave much histological assistance, and Messrs J. King and A. L. Wooding were responsible for the photographs.

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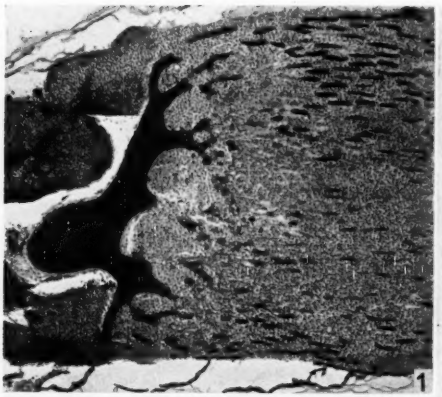
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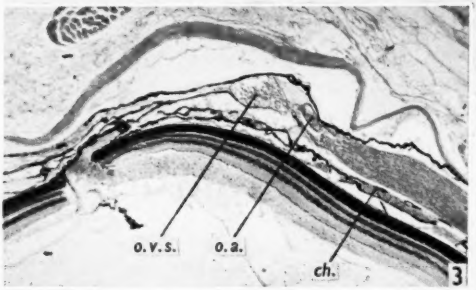
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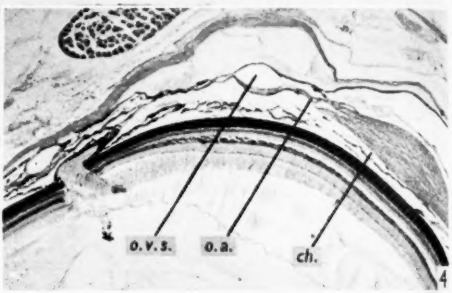
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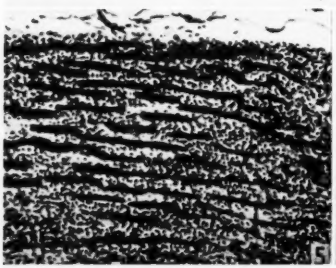
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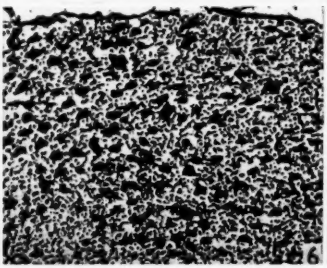
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EXPLANATION OF PLATE

Fig. 1. Branches of ophthalmic artery passing laterally into choroidal gland. Indian ink in plasma injection of dorsal aorta. × 70.

Fig. 2. Peripheral margin of choroidal gland. Indian ink in plasma injection. × 70.

Fig. 3. Back of eyeball of trout treated with amyl nitrite. × 35.

Fig. 4. Corresponding section to fig. 3, from trout treated with ergometrine tartrate. × 35.

Fig. 5. Choroidal gland to show alternation of injected (arterial) and non-injected (venous) capillaries. Indian ink in plasma injection. × 175.

Fig. 6. The same, in transverse section. × 175.

Fig. 7. Lentiform body. Neoprene injection. × 25.

KEY TO LETTERING IN TEXT-FIGURE AND PLATE

a.c.	arterial capillaries of choroidal gland
a.f.p.	arteries of falciform process
a.l.b.	arterial capillaries of lentiform body
ch.	choriocapillaris plexus
c.scl.	cartilaginous part of sclera
f.scl.	fibrous part of sclera
o.a.	ophthalmic artery and terminal divisions
o.n.	optic nerve
o.v.s.	ophthalmic venous sinus
r.v.	retinal vein
v.c.	venous capillaries of choroidal vein, opening into ophthalmic venous sinus
v.ch.v.	ventral choroidal vein

THE LOCAL ACTION OF CALCIFEROL AND OESTRADIOL ON BONE

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The application of the technique of intracerebral grafting to the study of the local action of the parathyroid and also of vitamin A on bone has already been described by the writer in previous publications (1948*a*, 1950). In the present paper the results obtained by the same method, using the two sterols calciferol (vitamin D_2) and oestradiol as chemical implants, are reported in full, following an earlier preliminary communication (1948*b*).

The two sterols are dealt with together in this paper as a matter of convenience only, since, although they are related chemically, their physiological properties are widely different. Like vitamin A they are sparingly soluble in aqueous solvents, which renders them suitable for the present technique.

THE LOCAL ACTION OF CALCIFEROL (VITAMIN D_2)

Introduction

The curative action of vitamin D in rickets has been the subject of much experimental work, and it is agreed that the major effect of the vitamin is to increase intestinal absorption of calcium and phosphate. Since, however, the present work is concerned with the action of calciferol when lying close to a bone surface and presumably present in high concentration, the literature of experimental overdosage is more relevant. McLean (1941) has pointed out that vitamin D appears to exert two distinct actions which occur at widely different dosage levels: first, the antirachitic action for which very small amounts are sufficient and, secondly, the 'calcemic' effect with several hundred times the antirachitic dose. He regards the rise of blood calcium which occurs in the latter case as due mainly to a release of bone minerals.

Kreitmair & Moll (1928) and Pfannenstiel (1928) reported that animals receiving massive doses of irradiated ergosterol often died with calcifications in the soft tissues. As Bills (1935) pointed out, however, the earlier preparations contained toxic derivatives and, moreover, the comparison of results was rendered difficult by lack of standardization of the products employed. Evidence of bone rarefaction resulting from high doses of irradiated ergosterol or of calciferol has frequently been reported (for example, by Baumgartner, King & Page, 1929; Shelling, 1930; Brown & Shohl, 1930; Shohl, Goldblatt & Brown, 1930; Harris & Innes, 1931; Shelling & Asher, 1932; Jones & Robson, 1933; Raab & Cohn, 1935). Most workers studied the rat, and in this species the precise effect on the skeleton was shown to depend on the absolute amounts and ratio of calcium and phosphate in the diet. Bauer & Marble (1932) reported similar results in young cats. It appears the bone resorption is only well marked if the vitamin dose is of the order of 10,000–20,000 times the antirachitic

dose and the calcium intake is low; with lower doses and more calcium and phosphate in the diet an actual increase of metaphysial trabeculae was observed, which probably accounts for the earlier findings of Collazo, Rubino & Varela (1929) and Selye (1932).

The question whether the vitamin produces bone resorption by a direct action on the bone tissue remains open, although there is considerable evidence against the view that the parathyroid is essential to the response (Harris, 1932; Asher & Jones, 1933; Jones, 1933). The bone lesions in hypervitaminosis D and parathyroid overdosage are said to differ in certain respects (Shelling, Asher & Jackson, 1933; McJunkin, Tweedy & McNamara, 1937). The view that the primary action of vitamin D is on the renal threshold for phosphate has also been advanced by Albright & Sulkowitch (1938). The present technique is designed to study the action of the vitamin when brought into close contact with bone tissue and thus to eliminate, to a large extent, the complicating effects of simultaneous actions on other tissues which are likely to occur in experiments on the intact animal.

Material and methods

All the experiments were performed on mice 10 days of age, and the grafts were removed 10–17 days after implantation. Supravital staining with neutral red, maceration and histological preparation were carried out as already described by the writer in a previous paper (1950).

Mice from two inbred lines, C57 Black and Glaxo Champagne, were used in these experiments. The earlier experiments were performed on C57 animals, and a sample of finely crystalline calciferol stored in air at 0°C. was used for the chemical implant. Champagne animals were utilized later on account of the higher fertility of the stock, but at the same time a change was made to a freshly crystallized sample of calciferol (sample II) sealed in nitrogen. Since the response to the implants made with the second sample on Champagne animals differed from that originally obtained on C57 animals using sample 1, further experiments were undertaken to check the response of both stocks, and to elucidate the possible influence of the state of division and freshness of the calciferol preparations on the results. The description of the work is therefore arranged in the following manner.

(1) C57 stock: (*a*) Calciferol sample I.
(*b*) Calciferol sample II.

(2) Champagne stock: (*a*) Calciferol sample II.
(*b*) Irradiated calciferol.

(3) Histological material, all of which is from C57 animals with implants of calciferol sample I.

(1) *Experiments on C57 Black stock*

(*a*) *Calciferol sample I*

The calciferol sample had the form of minute needle-like crystals. For implantation the powder was mixed with a solution of dried human fibrinogen which was then clotted with thrombin solution, and the powder was worked into a coherent mass from which pellets about 0·5 mm. in diameter were detached and fixed in the centre of the bone grafts.

Eight grafts were examined after maceration; five of them had been removed after 10 days and three after 17 days.

Of the five 10-day specimens, three showed definite resorption near the centre of the graft; here the bone was extremely thin and perforated, with minute, irregular holes. In the other two specimens there was some erosion and roughening of the bone surface, but it was less severe. The three 17-day specimens showed even more definite resorption which extended over much of the bone surface but was most extreme near the centre (Pl. 1, fig. 1). The somewhat diffuse character of the resorption found in these specimens contrasted with the sharp-edged single perforations which so often resulted from vitamin A implantation, as the writer has described elsewhere (1950). In some of the specimens the calciferol pellet was observed lying in close contact with the bone surface near the region of maximum resorption when the specimens were dissected from the host brain. Supravital staining was not employed in the examination of this batch of material.

(*b*) *Calciferol sample II*

This sample of calciferol consisted of angular fragments of varying size, and it was possible to select single fragments of comparable size to those employed for certain vitamin A grafts and for those with oestradiol. In view of the difference in the character of the resorption between vitamin A grafts and calciferol grafts, and because certain differences of response were noted when sample II was used with Champagne stock animals, it was thought that the state of division of the implant might influence the result, and four bone grafts from C57 animals were therefore set up which had been implanted with single fragments of calciferol sample II, carefully freed from adherent calciferol dust.

The grafts, which were removed after 14 days, differed considerably in structure from those described above. Only one specimen showed perforation, and this was very slight; the bones as a whole were thicker than in the previous specimens, but the central region was thinner than the periphery and was somewhat pitted and eroded (Pl. 1, fig. 2). The marginal part was sometimes distended by enlarged canals beneath the bone surface. Neutral-red staining of these specimens immediately after their removal from the host revealed a certain number of osteoblasts and osteoclasts, but these were not situated on the exposed bone surface as they are in vitamin A grafts, but were contained in deep pits or canals; nor did the osteoclasts appear to be more numerous in the region of the calciferol implant. Some parts of the bone surface adjacent to the calciferol stained a diffuse red and the outline of individual cells could not be distinguished. A feature which was found to be characteristic of calciferol grafts and which may have affected the cell distributions as shown in neutral-red preparations may be mentioned here; the layer of connective tissue covering the surface of the graft nearest the calciferol implant was noticeably adherent in comparison with vitamin A or other grafts, and it may be that in removing it after supravital staining some of the surface cells were pulled away.

In view of these results (which contrasted with those obtained with the more finely powdered calciferol sample I) some further experiments were performed using sample II made into pellets with fibrin after grinding as finely as possible. The particle size, however, was still larger than that of sample I and the form of the crystals was also different. The four grafts set up in this way were removed after

14 days; the macerated specimens were comparable in structure to those described already which were implanted with calciferol sample I, though resorption was in general less extreme than in the 17-day specimens of this latter group; the bones were thin in their whole extent, and there was well-marked diffuse erosion leading to perforation near the centre (Pl. 1, fig. 3). Neutral-red staining revealed only a few osteoclasts, and these were generally confined to pits and canals near the margins of the resorbed zone.

To sum up these experiments on C57 animals, which are admittedly too few to permit very secure conclusions on certain points, calciferol appears to produce definite local resorption of bone which is particularly pronounced if the preparation is in finely divided form. The character of the resorption is distinct from that produced by vitamin A acetate, and conspicuous aggregations of superficially situated osteoclasts do not seem to occur. Since the results were substantially the same with a sample of calciferol stored in air and with a recrystallized sample stored in nitrogen, provided the latter was used in finely divided form, it appears that contaminants due to oxidation and exposure to light are not likely to have played an important part in producing the observed results.

(2) *Champagne stock*

(*a*) *Calciferol sample II*

All the experiments on the Champagne stock were performed with calciferol sample II, since sample I was by this time used up. Eleven bones were implanted with single fragments and removed after 14 days. All of them showed some roughening and pitting of the surface, but it was diffuse, and only in three cases was it noticeably more severe in the central region occupied by the calciferol (Pl. 1, fig. 4); even in these, however, perforation had not occurred. Some of the specimens showed the same swollen canals in the bone which were noted in certain C57 grafts, and supravital staining revealed osteoclasts and osteoblasts within them.

Eight grafts were performed with the calciferol ground into finer form and prepared as pellets in fibrin. Evidence of resorption was on the whole more marked than in bones implanted with single fragments (Pl. 1, fig. 5); in four of them it was definitely localized in the central region, but in no case was perforation extensive, and the degree of resorption was undoubtedly less than in the corresponding C57 grafts. The results suggest that there may be a real difference in the response of the two strains to calciferol under these particular conditions.

(*b*) *Irradiated calciferol, sample II*

As a further check on the possibility that contaminants in the calciferol preparations might have influenced the results a number of grafts were set up, using pellets of sample II after irradiation in ultra-violet light. Small quantities of the powder were spread out in a glass dish covered with a quartz plate and exposed for 15–20 min. to the light from a quartz-mercury burner, at a distance of approximately 3 in. During irradiation the temperature of the dish was raised to above 40°C., and the powder became slightly yellow. According to Bills (1939) the relative amounts of the derivatives which result from irradiation of ergosterol or calciferol vary according to the wave-length of the light, the nature of the solvent and other factors, so that the

final composition of the material produced by the present crude procedure is uncertain. If, however, contaminants played any part in the results so far described they were likely to have accumulated as a result of exposure to sunlight in the presence of air, in so far as they were not already present in the original samples.

Six grafts were performed with this irradiated material made into pellets, and grafts with unirradiated powder were performed on litter-mates for comparison; the latter have already been dealt with. The bones carrying irradiated material showed, after 14 days, quite definite resorption in the central region, and perforation was more frequent and generally more severe in them than in any other grafts on Champagne stock animals (Pl. 1, fig. 6). The resorption was certainly more severe than in the controls with unirradiated material. Although a more extensive series of experiments would be needed to verify this result, it seems probable that irradiation may produce derivatives of calciferol capable of intensifying resorption. It is not likely, however, in view of the experiments already described in previous sections, and the fact that irradiation of the samples was much more severe than could occur from mere periodic exposure to daylight, that such an action of contaminants need be seriously considered in assessing the results as a whole.

(3) *Histological material*

The material consists of nine specimens, all from C57 animals implanted with calciferol sample I. The grafts were removed 14 days after the operation with the surrounding brain tissue, fixed in Zenker acetic, double embedded and cut serially through the region of the calciferol pellet.

In six of the grafts the pellet had retained its position near the centre of the bone, while in three it had become displaced towards the skull surface. There was evidence of resorption in all nine grafts, and in those with undisplaced pellets the adjacent bone was thin and often perforated in one or more places (Pl. 2, figs. 12, 13). This thin bone was generally paler staining than the thicker bone on either side, and much of it was undoubtedly a new deposit. Osteoclasts occurred on the surface nearest the calciferol, but only in one specimen were they at all numerous opposite the pellet itself (Pl. 2, fig. 13); more frequently they occurred at the zone of junction between the thick and thin bone. It was also a peculiar feature of calciferol grafts that osteoclasts could be found on the surface farthest from the pellet, that is, the convex aspect, whereas in most vitamin A and parathyroid grafts there was a layer of active osteoblasts lining this surface and evidently laying down bone which was in process of resorption by numerous osteoclasts restricted to the opposite concave aspect. Resorption and new bone formation are both in evidence in the vicinity of calciferol pellets but they seem to be less active than in parathyroid and vitamin A grafts, and less uniformly distributed over the whole of the area; large basophil osteoblasts can be seen (Pl. 2, fig. 13) in association with new bone, but they occur only in patches, while a considerable part of both surfaces of the resorption zone is lined with fibroblasts which may form quite a thick layer (Pl. 2, fig. 12). It would require further study on earlier stages of grafts to elucidate the mode of action of calciferol with certainty, but it appears probable that active resorption is not long maintained opposite the pellet and becomes confined to the zone around it. The possibility that a general toxic effect which inhibits cellular activity to some

extent complicates the picture of resorption and osteogenesis seems worth consideration, although there is no intense inflammatory reaction or signs of widespread necrosis.

In two of the specimens with displaced pellets the spongy bone at the edge of the graft where it lay in the scar tissue of the insertion hole was clearly being eroded by osteoclasts, while in the third the bony thickening of the graft which generally occurs along this edge was absent although osteoclasts were not found, suggesting a suppression of bone deposition due to the calciferol. The structure of the calciferol pellet is illustrated in Pl. 2, fig. 13. The spaces left by the crystals were often sharp-edged, but the fact that in some specimens they were distinctly smaller on the side nearest the bone surface suggests that considerable solution or destruction of the calciferol may have occurred and have been most intense on this aspect. The crystal spaces were surrounded by the cytoplasm of multinucleate giant cells, spread out over them as a thin, often foamy layer. This cytoplasm was generally acidophil, and sometimes a layer of brightly acidophil granules was distinguishable in immediate contact with the crystal surface; the same feature was sometimes noted in osteoclasts, and with the simple staining procedure (haematoxylin-orange G-erythrosin) here employed often no distinction could be made between these giant cells and osteoclasts on the neighbouring bone. However, supravital staining with neutral red never showed the deep, granular coloration characteristic of osteoclasts in association with the crystals. Apart from the giant cells, strands of cells resembling fibroblasts traversed the pellet, and there were also scattered cells, the nuclear staining of which was similar to that of certain elements, presumably glia, situated in the nearby brain tissue. Capillaries could be detected on the surface of the pellet adjacent to both brain and bone, but very few were seen within the pellet. Some of the vessels could be traced to the host pia, while others may have been derived from the scar tissue in the host skull.

On the whole the histological findings are consistent with the observations on macerated and supravitally stained material. The scarcity of osteoclasts close to the calciferol in the latter appears to be due to an actual deficiency of these cells, and the conspicuous layer of fibroblasts on the graft surface no doubt accounts for the difficulty in removing the superficial layer of the grafts. No histological material, however, is available of bones showing the peculiar swollen appearance which occurred in grafts to Champagne stock animals.

Grafts of ergosterol and cholesterol

In order to gain a clearer insight into the specificity of the local action demonstrated for calciferol, the two allied sterols, cholesterol and ergosterol, were used as implants, pellets being made from finely powdered samples. Neither of these substances is known to lead to skeletal resorption, nor, indeed, to any disturbance of the skeleton when administered to the intact animal. Ergosterol is, of course, the substance from which calciferol is obtained by irradiation, but is not a normal metabolite, while cholesterol is widely distributed in the tissues and may be involved in the synthesis of some sterols.

Cholesterol

The eight bones implanted with cholesterol for 14 days and examined after maceration showed hardly any features which do not also occur in pieces of parietal

bone grafted without chemical implants. The surface was somewhat irregular (Pl. 1, fig. 7), and in three specimens there was a slight depression marking the position of the pellet. The thinnest areas of the bone were, however, quite smooth and comparable in structure with ungrafted bones of 10-day animals, so that the irregularities were due in all probability to irregular secondary deposition rather than to resorption, and this is borne out by the two specimens which were also supravitally stained and showed very few osteoclasts and only occasional patches of osteoblasts on the bone surface. Five grafts were prepared histologically, and one is illustrated in Pl. 2, fig. 14. The cholesterol particles were considerably larger than the crystals of calciferol sample I. The pellets lay closely applied to the bone surface. The graft bone was somewhat thicker near its edges than in the centre near the pellet, and the edge nearest the skull wound had a spongy structure. Closer examination showed that both surfaces, which were lined with osteoblasts, consisted of a layer of new bone with living osteocytes laid down over the original graft bone and separated from it by cement lines. There was no evidence of resorption in the region of the cholesterol, and it seems likely that the depressions occasionally noted in this position were due mainly to the pellet acting as a mechanical obstacle to new bone formation.

Ergosterol

The ergosterol preparation was a freshly recrystallized sample considered to be 95 % pure. Eight grafts were performed with pellets of this material on animals of the C57 stock, and they were removed after 14 days. Examined after maceration, the specimens showed the same kind of irregular thickening as cholesterol specimens, and though in some cases it was rather more severe (Pl. 1, fig. 8), there was no evidence of local resorption. Supravital staining revealed some osteoclasts either dispersed over the surface nearest the implant or aggregated in groups, the position of which, however, was not coincident with the site of the chemical implant. Osteoclasts were perhaps more numerous than in cholesterol grafts.

Discussion

The work demonstrates that calciferol can stimulate resorption of bone under conditions which appear to exclude any mechanism but a local action on bone tissue; thus the effect was fairly sharply restricted to the vicinity of the implant, and there were no symptoms of generalized hypervitaminosis in the host animals. The effect also has a certain specificity, for the control material shows that mechanical factors cannot be of primary importance and that the response is not obtainable by sterols in general. The facts strongly suggest that the skeletal resorption which has often been reported in animals given sufficiently high doses of irradiated ergosterol or of calciferol may also be due to a direct action on the bones, though the influence of other factors under these conditions cannot be excluded. Somewhat indirect evidence of a local action of vitamin D on the skeleton in healing rickets has been advanced by Morgareidge & Manly (1939) and by Greenberg (1945) on the basis of radioactive tracer studies, but whether this action of smaller doses is in any way related to the resorption obtained with much higher doses remains unknown. The grafting technique has the limitation that it provides no quantitative information as to the local concentration of calciferol at the site of resorption; the histology

suggests that only a small proportion of the substance goes into solution during the course of the experiment. Whether, however, as appears to be the case with vitamin A, any chemical transformation of the calciferol occurs locally in the tissues, thereby perhaps inactivating some of it, is not at present known.

THE LOCAL ACTION OF OESTRADIOL

Introduction

The action of the sex hormones on the skeleton has been comprehensively reviewed by Gardner & Pfeiffer (1943). The hyperossification of the medullary cavities of the long bones, first reported as a physiological occurrence in birds by Kyes & Potter (1934) and later produced in male pigeons with oestradiol benzoate by Gardner & Pfeiffer (1938) and by the same authors (1939) in mice, is most relevant to the present study. Gardner & Pfeiffer found that in mice the medullary cavities of the long bones became filled with trabeculae if 100–1000 i.u. were given weekly for as long as one year; similar changes occurred in other parts of the skeleton while the pubic bones underwent resorption. Gardner (1940) obtained these effects with a variety of natural and synthetic oestrogens, and showed strain variations in the rapidity of response. Sutro (1940), relying on radiographic examination, reported changes in the skull which may have involved resorption in mice given 100–150 r.u. weekly. Silberberg & Silberberg (1941) stated that sufficiently prolonged treatment led, in the mouse, to resorption of the excess bone; they described (1939) the medullary hyperossification in the guinea-pig as being due to transformation of marrow reticulum cells to osteoblasts, which is consistent with Bloom, Bloom & McLean's (1941) work on pigeons. Day & Follis (1941) attributed the hyperossification in young rats to simple failure of normal resorption, but this seems improbable since Simpson, Kirbrick, Becks & Evans (1942) found that one-fifth of the marrow cavity of young rats might become filled with trabeculae after 16 days' treatment. Pfeiffer (1948) was unable to show any effect of oestrogen administration on the development of bone from fragments of marrow implanted into the anterior chamber of the eye.

Kirbrick, Simpson, Becks & Evans (1942) claimed that hyperossification was not produced in oestrogen-treated hypophysectomized rats, which is contrary to Gardner's report quoted by Pfeiffer (1948). The absence of a response after hypophysectomy would not necessarily indicate the prime role of pituitary secretions, but might simply mean that the response of the bones to oestrogens is in part dependent on normal pituitary functioning.

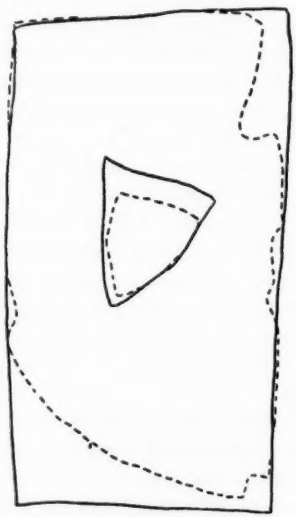
The local action of oestrogens on other tissues has been reviewed by Speert (1948), and highly localized responses are reported in the case of vaginal epithelium, uterine mucosa, nipple, comb and feather follicles.

The present experiments were performed with small chips broken from a fused tablet of oestradiol. Twenty-eight grafts were performed with pieces of parietal bone, thirteen being with bone from a female grafted to a female host, eight with bone from a male to a male host, two from female bone to a male host, and five from an animal of unknown sex to a male host. The majority of experiments were on C57 animals, a strain which is reported to be relatively sensitive to the skeletal effects of oestrogens, but the Champagne stock, the sensitivity of which has not been recorded, was also

used. Further experiments were also performed with the shafts of femora. All grafts were left *in situ* for 14 days.

Grafts of parietal bone

The macerated specimens can be dealt with very briefly, since they showed, in fact, no features which are not also to be found in grafts without chemical implant or with implants of inactive substances. In supravitally stained specimens patches of osteoblasts and very occasional osteoclasts were seen, but their distribution bore no relation to the site of the oestradiol. In only thirteen of the twenty-eight grafts was the residue of oestradiol found when the graft was removed, and it had obviously decreased considerably in size and the originally sharp corners of the fragment had become rounded. This suggested that in some of the grafts the oestradiol had entirely disappeared by solution, thus curtailing the duration of its action on the bone. Six grafts were therefore performed with flat chips of oestradiol approximately two or three times as large as those originally used, and to avoid too great a proportion of the bone surface being covered, the size of the piece of parietal bone was also increased. Examined by maceration after 14 days the grafts were distinctly different from those already described. The bone was unusually thick and swollen by large canals and spaces lying below the surface (Pl. 1, fig. 9); this condition was comparable with that described in grafts carrying large single fragments of calciferol, which suggests that the size of the fragment rather than a specific chemical effect is the essential factor in both cases.



Text-fig. 1. Scale drawing of a graft of a large oestradiol fragment. The outlines of the bone graft and oestradiol implant at the start of the experiment in solid line and the outline 14 days later in broken line. ×50.

The area of the implanted fragment both at the time of implantation and of removal was recorded by drawing on to graph paper under the ocular graticule in all these six specimens, and in Text-fig. 1 the outline of one of them at the time of grafting and at

removal is reproduced. The area was found to have decreased by three-quarters to one-third of its initial size.

Before turning to a description of the histological material, mention may be made of certain grafts which are described in the writer's work on vitamin A (1950). In these about 50% of oestradiol was mixed with powdered vitamin A, since it was thought that the oestrogen might manifest some activity by inhibiting the marked resorption obtained with vitamin A alone; no evidence of such an effect was observed, however.

Histological material

Twelve specimens, all from C57 animals, and carrying small chips of oestradiol, were examined by serial sectioning through the grafts, which were fixed *in situ* with the surrounding brain tissue. The material included various combinations of sex of host and donor, but these variations do not appear to have significantly affected the results and need not be detailed. In only four specimens was the oestradiol residue found in the sections; in three it lay close to the bone surface near the centre of the bone graft, and in the fourth it was somewhat displaced towards the skull wound (Pl. 2, fig. 16). The bone graft showed a fairly regular and compact layer of new bone along its concave aspect both in the vicinity of the oestradiol and elsewhere; this surface was lined with osteoblasts which in some places were large and quadrangular and in other places flattened (Pl. 2, fig. 15). There was thus no evidence of any local action of the oestradiol. The oestradiol residues themselves (Pl. 2, figs. 15, 16) showed an interesting structure; they were surrounded by a layer of acidophil macrophages often with a somewhat radial arrangement, which were closely applied to an unstained, homogeneous capsule forming the outer layer of the fragment. This capsule was, no doubt, the 'ghost' originally reported by Folley (1942), which forms round subcutaneous implants of various steroids.

Grafts of femora

The negative results obtained with parietal grafts, and the fact that the effects of oestrogens in promoting hyperossification are most marked, according to previous reports, in the marrow cavities, suggested that material from a long bone should be tested by the grafting technique.

The grafts were performed in the following way. The femur was removed into saline and, after the muscles had been dissected off as completely as possible, the two ends of the bone were removed with a scalpel at the level of the metaphysis; the resulting cylinder of bone was then cut into two pieces longitudinally, and in the majority of cases the marrow tissue was removed, apart from fragments adherent to the endosteum which cannot be detached without risking injury to the bone. A fairly large fragment of oestradiol was placed in close contact with the endosteum half-way along the shaft and was attached with fibrin in the usual way. The graft was then thrust vertically into the host brain. In many cases the two halves of the same femur were grafted into the opposite hemispheres of the same host, one carrying cholesterol and the other oestradiol. Twenty-four grafts were performed with oestradiol; seventeen of these were grafts in which the marrow was removed, and included grafts from female animals to female hosts and also from males to male hosts. The

remaining seven were grafts with the marrow removed, and these included some from females to females, males to males and females to males. Seven grafts were performed with cholesterol implants, the marrow being removed; these included grafts from females to females and from males to males. Seven cholesterol grafts were also performed with the marrow intact, and included some of females to females, males to males, and females to males. Finally, there was one graft, from a female to a female, in which no chemical implant was made. The experiments are summarized in Table 1.

Table 1

		Nature of chemical implant		
		Oestradiol	Cholesterol	None
	Litter No.	Type of graft; Donor–Host		
Marrow removed	1	♀–♀	—	—
	2	♂–♂	—	—
	3	♀–♀	—	♀–♀
	4	♀–♀	♀–♀	—
	5	♂–♂	♂–♂	—
Total number of grafts		17	7	1
Marrow not removed	6	♀–♀	♀–♀	—
	7	♂–♂	♂–♂	—
	8	♀–♂	♀–♂	—
Total number of grafts		7	7	0

Animals of both C57 Black and Champagne stock were used in this series of experiments. The grafts were examined by maceration and no histological material was prepared.

Most of the specimens showed only slight evidence of new bone deposition on the endosteal surface, nor was there any sign of resorption in the vicinity of the chemical implant (Pl. 1, fig. 10). In five oestradiol and three cholesterol specimens, however, the appearance was very different (Pl. 1, fig. 11). The whole marrow space was filled with a dense mass of spongy bone which partially surrounded the chemical implant but was by no means restricted to its vicinity. No reason for the sporadic occurrence of this excessive bone deposition is apparent; it sometimes occurred in both halves of the same femur, the one bearing oestradiol and the other cholesterol, which had been grafted to the same host, but in another instance it occurred in one and not the other. The phenomenon could not be correlated with the nature of the chemical implant, the sex of donor or host or whether or not the marrow tissue was removed.

Discussion

The experiments failed to show any local action of oestradiol on bone, even in the case of the femoral shaft. It might perhaps be argued that in the latter case the exclusion of the metaphysial spongiosa would account for the failure; however, the literature suggests that the endosteal surface of the shaft and also the marrow participate in hyperossification, and this second possibility has also been taken into account in the experiments. The fact that the grafts were only of 14 days duration may be a more serious criticism, since most authors maintained a high dosage for several months. Evidence of hyperossification has not been reported earlier than 2 weeks after oestrogen treatment, but, on the other hand, workers have not been

concerned to detect the very earliest signs of an effect, and with a technique such as the present one, where a high concentration of oestrogen may be supposed to act on a very restricted region of the bone, comparatively slight changes should be readily detectable. A number of fragments of oestradiol of the same range of size as those used for grafting were found to have an average weight of 0·024 mg. which would contain 240 i.u. From a consideration of the decrease of projected area of the implants and their shape, it is reasonable to assume that something of the order of 100 i.u. was absorbed in 14 days. In an animal of 5·0 g. weight this would imply a dosage which would be equivalent to a dosage of 200 i.u. per week to a 20 g. adult, while the dosage range to produce hyperossification is of the order of 500–1000 i.u. per week by weekly injection. However, the method used in the grafts is more comparable to a tablet implantation, which should be more efficient than injections since a continuous dosage is in effect maintained; moreover, the oestrogen is held close to the bone, and is presumably in a higher concentration at the bone surface than it could be expected to reach as a result of injections or the solution of a subcutaneous pellet. While there are too many unknown factors to allow anything but very tentative conclusions, it appears that the local dosage may well have been adequate to produce an effect on the bone graft if a local action is in fact possible. It is important to note, however, that no obvious effects of oestrogen overdosage, such as failure of growth, were noted in the host animals, in so far as they were not noticeably different in size from their control litter-mates at the time of killing, and in a number of cases in which the size of the uterus or seminal vesicle was compared with that of controls, no difference was observed, and this is perhaps surprising in view of the amount of oestrogen which appears to have been absorbed.

SUMMARY

1. Calciferol in the form of small pellets was attached to pieces of parietal bone from 10-day-old mice and grafted intracerebrally to litter mate animals. After 14 days a diffuse resorption of bone, often leading to perforation, was observed. This effect was not obtained with ergosterol or cholesterol.

2. It was shown by histological sectioning that this resorption was accompanied by osteoclasts, but these cells were less conspicuous in specimens stained supravitally with neutral red than they were in grafts of vitamin A. A certain amount of new bone formation also occurred at the site of resorption.

3. The resorption was more intense if the calciferol was in finely divided form. Differences between two strains of mice in the degree of response were noted, and some evidence was found that irradiation of the calciferol in ultraviolet light increased the resorption it produced.

4. Similar experiments were performed with oestradiol, using both pieces of parietal bone and also portions of the femoral shaft. No local action of the oestrogen could be demonstrated.

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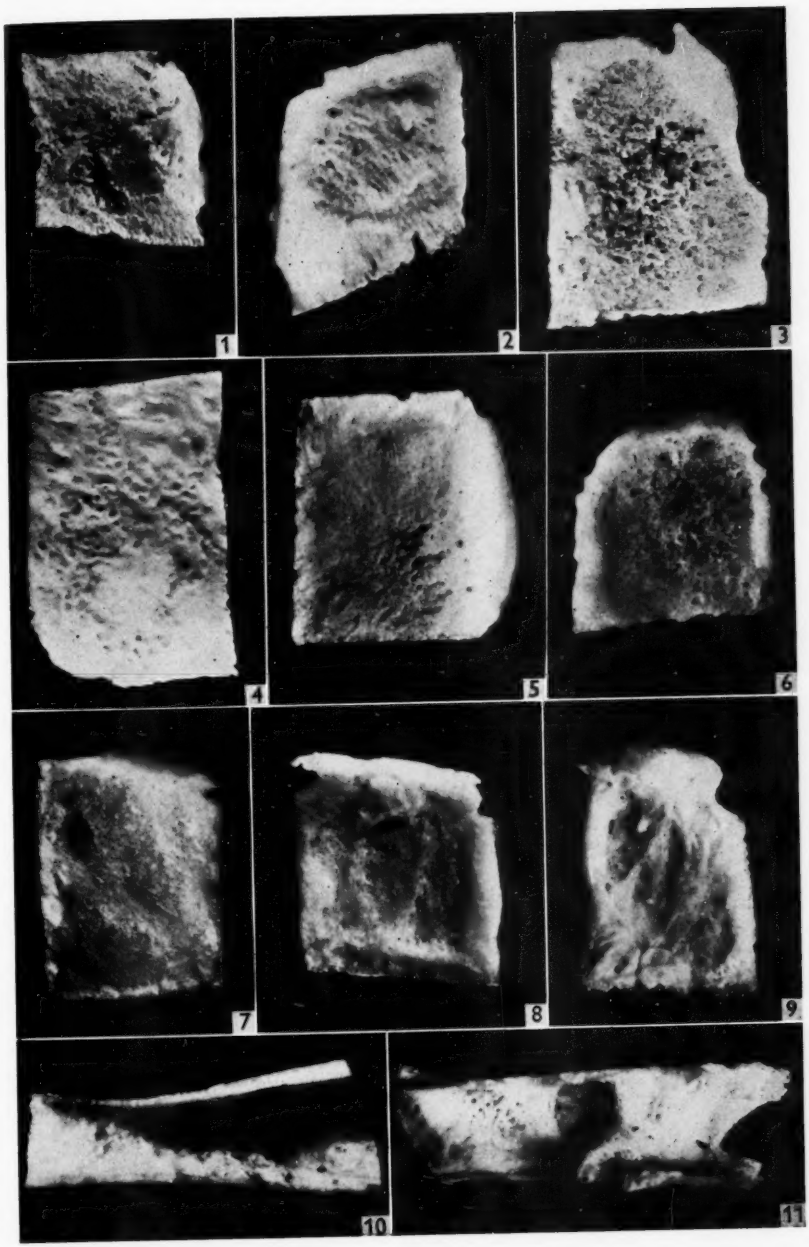
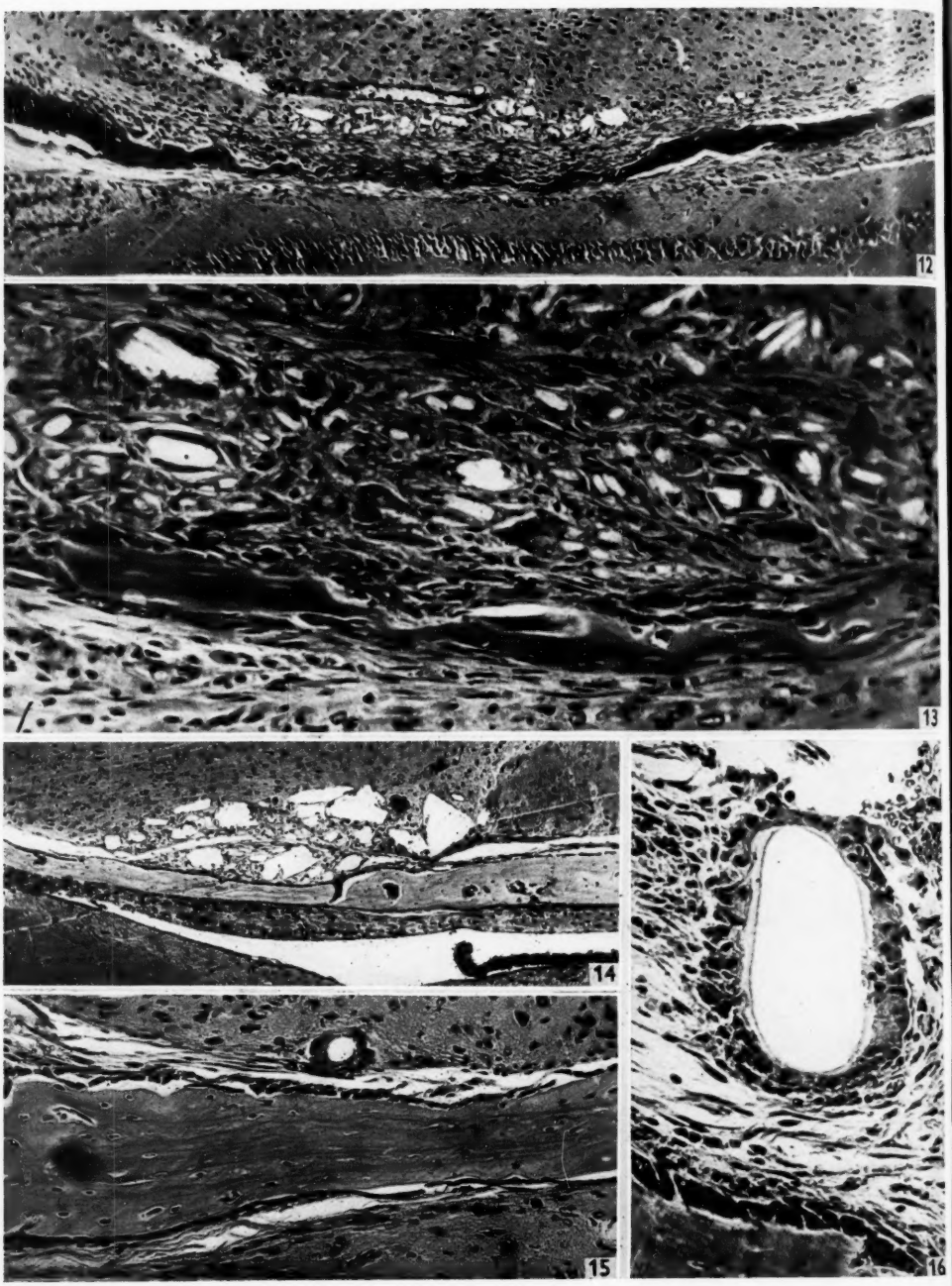


BARNICOT—Local action of calciferol and oestradiol on bone



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EXPLANATION OF PLATES

PLATE 1

Macerated specimens.

Fig. 1. Graft implanted with calciferol sample I; removed after 17 days; C57 stock. There is diffuse resorption leading to well marked perforation in the position occupied by the calciferol.

Fig. 2. Graft implanted with a single large fragment of calciferol sample II; removed after 14 days; C57 stock. The central region is somewhat roughened and is thinner than the swollen margins.

Fig. 3. Graft implanted with powdered calciferol sample II; removed after 14 days; C57 stock. Diffuse resorption which is almost as pronounced as with calciferol sample I.

Fig. 4. Graft implanted with a single fragment of calciferol sample II; removed after 14 days; Champagne stock. The central area is somewhat pitted; the entire bone is unusually thick.

Fig. 5. Graft implanted with powdered calciferol sample II; removed after 14 days; Champagne stock. The greater part of the bone is thin and there is a slight multiple perforation in the region occupied by the calciferol.

Fig. 6. Graft implanted with powdered calciferol sample II which had been irradiated; removed after 14 days; Champagne stock. There is a large area of resorption in the top right-hand corner, in the centre of which perforation has occurred.

Fig. 7. Graft implanted with powdered cholesterol; removed after 17 days; C57 stock. There is irregular thickening of the surface with a slight depression marking the site of the cholesterol just to the right of the centre.

Fig. 8. Graft implanted with powdered ergosterol; removed after 14 days; C57 stock. There is irregular thickening and a depression near the centre marks the position of the ergosterol pellet, but there is no definite evidence of resorption.

Fig. 9. Graft implanted with a large fragment of oestradiol; removed after 14 days; Champagne stock. The bone is thick and swollen and the central area shows well marked but irregular bone deposition.

Fig. 10. The endosteal surface of a bisected femur which had been grafted for 14 days with an oestradiol fragment attached half-way along its length. Slight irregular bone deposits can be seen, particularly at the margins of the shaded area. The site of the oestradiol implant shows no special features.

Fig. 11. Specimen implanted with oestradiol as in fig. 10. The entire marrow space is filled with spongy bone, within which appears the space occupied by the oestradiol implant.

PLATE 2

Fig. 12. Graft of powdered calciferol sample I to a C57 animal. The bone of the graft shows a marked contrast in staining and thickness between the very thin central region opposite the calciferol pellet and the thicker regions on either side. Note the thick layer of fibroblasts between the pellet and the bone surface, and also the ragged outline of parts of the bone surface. Haematoxylin-orange G-erythrosin. ×123.

Fig. 13. Calciferol pellet and adjacent bone. The bone graft in this specimen showed multiple perforations. Crystal spaces of various sizes, surrounded by giant cells, can be seen in the pellet. Osteoclasts occur on the bone surface nearest the pellet and one is also seen on the opposite surface. On the left-hand side the dark-staining bone with empty lacunae is bone of the original graft, while on the right-hand side a thin layer of paler new bone, with living osteocytes, has been deposited on its upper surface. Haematoxylin-orange G-erythrosin. ×380.

Fig. 14. Graft of powdered cholesterol to a C57 animal. The large angular crystal spaces of the pellet are visible lying close to the bone which shows no evidence of resorption. Both surfaces are lined with osteoblasts but the newly deposited layers of bone are not distinguishable on the photograph. Haematoxylin-orange G-erythrosin. ×92.

Fig. 15. Graft of oestradiol fragment. The small residue of oestradiol with its layer of ghost material and attached macrophages lies close to the surface of the bone graft. The original graft bone with empty lacunae is clearly demarcated from layers of new bone with living osteocytes laid down on both its surfaces, which are lined with large osteoblasts. Haematoxylin-orange G-erythrosin. ×210.

Fig. 16. An oestradiol implant lying a short distance from a bone graft (below). The capsular 'ghost' is clearly seen together with the surrounding macrophages. Note the rounded form of the originally angular fragment of oestradiol. Large quadrangular osteoblasts are seen on the adjacent bone surface. Haematoxylin-orange G-erythrosin. ×350.

OBSERVATIONS ON THE STRUCTURE AND PROPERTIES OF THE PLANTAR CALCANEO-NAVICULAR LIGAMENT IN MAN

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The plantar calcaneo-navicular ligament in man unites the anterior margin of the sustentaculum tali to the tuberosity of the navicular. It is a broad, flat, yellowish band approximately 2–3 mm. thick, 25 mm. long, and 12 mm. wide at its mid-length in the adult. Looked at from above its general shape is rhomboidal with its longer side on the tibial aspect. It forms the greater part of the inferior surface of the joint at the head of the talus, converting the anterior talo-calcanean joint and the talo-navicular joint into a partial socket for the head of the talus. The upper surface of the ligament is covered (at least in its lateral part) with articular cartilage. It is commonly known as the 'spring' ligament, and is presumed, by its position, to control the descent of the head of the talus. No opinion is expressed in standard text-books of anatomy or histology as to its physical properties, and its synonym leads frequently to the view that it is elastic in nature and so allows descent of the head of the talus in various movements of the foot. The present observations have been made in order to throw further light upon this question.

MATERIAL

For determining whether the head of the talus in the human foot does in fact descend, standard radiographs taken by a method described elsewhere by Venning & Hardy (1950) were used. It was found that this method made possible the production of successive radiographs of any individual foot, from which measurements could be taken within certain definite limits of accuracy. It was found, for instance, that, in measuring the 'arch-height' dimension (see below), in an average measurement of 45 mm. a standard deviation of $\pm 1{\cdot}5$ mm. occurred. This deviation takes into account the errors due to mensuration, to the optical limitations of the skiagraphic method, and to variations in the subject's foot from time to time. From these films measurements were also made of the amount of separation between the bony attachments of the spring ligament and of the long (vertical) axis of the navicular.

The association between histological structure and physical properties of ligaments was investigated in the cat. Of a number of ligaments examined two were found suitable for illustrating this association: the ligamentum patellae and the ligamentum flavum. These ligaments had the great advantage that they could be rapidly removed from the animal together with their bony attachments, a fact which made them easy to handle.

Four fresh specimens of human plantar calcaneo-navicular ligament were used to study the histological structure of the spring ligament itself.

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Radiography of human foot. A series of lateral (medio-lateral) radiographs was taken of each foot of ten subjects (students under 30 years of age; five of each sex) under the following three different conditions:

(*a*) Standing upright, with knee extended, and the whole body weight borne on one foot ('standard' position).

(*b*) Standing with the whole body weight borne on one foot with knee flexed, and trunk, thigh and leg internally rotated as far as possible (a one-legged, squatting position). As the foot is fixed by having its inferior surface applied to the ground and by bearing the whole body weight through that area of contact its relation to the horizontal is barely altered. It is however subjected to what may be called 'passive eversion and extension'.

(*c*) Standing as described in (*a*), but exercising an active contraction of the tibialis posterior muscle. This was a practised movement often requiring some minutes of precept and example before it was mastered. The foot was kept in a relatively stable position as before by the weight-bearing contact of its inferior surface with the ground, and was subjected to what may be called 'active inversion and adduction'. The tibialis posterior (the function of which is described by Duchenne (1867) as the approximation of the tuberosity of the navicular to the tibial malleolus) is here acting between two relatively fixed points—the standing leg and the weight-bearing foot—so that its force will be exerted upon its points of deflexion, behind and beneath the tibial malleolus and beneath the head of the talus, with the spring ligament interposed.

Measurements of three dimensions on each set of twenty radiographs thus obtained were made as follows:

(i) The distance from the lowest point on the head of the talus to a base-line (Pl. 1, fig. 1, *AB*) joining the lowest point of the sesamoids of the first metatarso-phalangeal joint to the lowest point of the calcaneum (hereinafter referred to as the 'arch-height' dimension; Pl. 1, fig. 1, *CD*).

(ii) The apparent length of the plantar calcaneo-navicular ligament, measured as the distance between a point midway on the anterior margin of the sustentaculum tali and a point midway down the posterior border of the tuberosity of the navicular (hereinafter referred to as the 'spring-ligament' dimension; Pl. 1, figs. 2 and 3, *EF*).

(iii) The apparent length of the long (vertical) axis of the navicular itself (Pl. 1, figs. 1, 2 and 3, *GH*).

Tables 1, 2 and 3 give the results of these measurements as the mean and standard deviation of each group, the mean differences between the 'standard' position, and those of 'passive eversion' and 'active inversion' respectively, with a *t*-test for the significance of the differences observed between each two groups.

Table 1. '*Arch-height*' (*mean of measurements of twenty radiographs*) *under conditions described in the text*

Position	Mean measure (mm.)	Mean difference (mm.)	S.D. of differences (mm.)	Value of '*t*'	Probability ($f=19$)	Significance
(*a*) Standard	45·0	(*a* – *b*) 7·6	±3·8	9·02	<0·001	Significant
(*b*) 'Passive eversion'	37·4	(*c* – *b*) 10·0	±4·5	10·11	<0·001	Significant
(*c*) 'Active inversion'	47·4	(*c* – *a*) 2·4	±1·7	6·48	<0·001	Significant

Pl. 1, figs. 1–3 show typical radiographs of the right foot of one subject in each of the three positions described.

Table 2. *'Spring-ligament length' (mean of measurements of eighteen* radiographs)*

Position	Mean measure (mm.)	Mean difference (mm.)	S.D. of differences (mm.)	Value of '*t*'	Probability ($f=18$)	Significance
(*a*) Standard	19·7	($a-b$) 0·5	±1·3	0·60	>0·5	Not significant
(*b*) 'Passive eversion'	19·2	($c-b$) 0·7	±1·0	0·74	>0·4	Not significant
(*c*) 'Active inversion'	19·9	($c-a$) 0·1	±1·0	0·19	>0·8	Not significant

* One case showed bilateral absence of the sustentaculum tali, so that no measurement could be made.

Table 3. *Long axis of navicular (mean of measurements of twenty radiographs) under same conditions as Tables* 1 *and* 2

Position	Mean measure (mm.)	Mean difference (mm.)	S.D. of differences (mm.)	Value of '*t*'	Probability ($f=19$)	Significance
(*a*) Standard	33·7	($b-a$) 3·4	±1·5	5·33	<0·001	Significant
(*b*) 'Passive eversion'	37·1	($b-c$) 6·3	±2·5	8·53	<0·001	Significant
(*c*) 'Active inversion'	30·7	($a-c$) 3·7	±1·8	4·85	<0·001	Significant

PHYSICAL PROPERTIES OF LIGAMENTS

Classical work on the breaking strain of human and animal ligaments and tendons has been done by Cronkite (1936), and on the elasticity of small strips of fascia lata by Gratz (1931). The object in the present investigation was to find two ligaments of compact formation with entirely different functions, and for the reasons outlined above the ligamentum patellae and the ligamentum flavum of the cat were used. The function of the former, which is a tendon developmentally and morphologically but nevertheless a collagenous structure uniting two bones, is clearly to transmit the force exerted by the contraction of the quadriceps femoris to the tibia, via the patella. The function of the ligamenta flava is, however, very different; they may be said to exert a controlling effect upon the neural arches in flexion of the spine, and presumably tend to restore them to apposition with one another against the force of active flexion.

These two ligaments were selected to determine the relationship between histological structure and physical properties, in particular the occurrence of elasticity, which for the purpose of this paper is defined as the ability to regain the original length after stretching.

Only brief mention need be made of the work on these ligaments, and their characteristic histology is well known.

The ligamentum patellae, when subjected to longitudinal loading, breaks before any visible stretching occurs. In one instance (the ligament of a 14 lb. cat) the breaking load was 56 lb. on the fresh material used. This ligament is entirely collagenous in structure. The ligamentum flavum, on the other hand, stretches to approximately 140 % of its original length under comparatively small loads and regains its original length immediately the load is removed. Histologically it contains a very large amount of elastic tissue.

The four plantar calcaneo-navicular ligaments were obtained from four female subjects aged 14 years (death from liver disease), 26 years (death from obstetric shock), 42 years (death from stem-cell lymphoma) and 59 years (from a leg amputated

below the knee for diabetic gangrene of the great toe). In each case the specimens were fixed, within 24 hr. of death or amputation, in 10 % formol-saline. Longitudinal sections were made, after fixation under tension, by mounting in wax and celloidin. Haematoxylin and van Gieson's stains were used to show the general histological structure of all the ligaments, and Weigert's elastin stain was used for the specific purpose of determining whether elastic tissue was present.

Owing to the fact that elastic ligaments lose their characteristic physical properties within an hour or so after removal from the body it was not possible to perform valid tests of loading upon the human material. It is not, however, without interest to observe that it was impossible to produce any perceptible stretching in the one human 'spring ligament', examined within 5 hr. of amputation, under a load of 56 lb. applied in the manner described above.

Histologically the human plantar calcaneo-navicular ligament (Pl. 2, figs. 4, 5) consists of dense bundles of collagen fibres, partly parallel in their arrangement but partly interlacing in appearance. In the substance of the ligament no elastic fibres could be detected by Weigert's elastin stain, although elastic tissue was clearly seen in the walls of blood-vessels and to some extent in the associated peri-articular connective tissue.

CONCLUSIONS

It is concluded from radiographic evidence that there is an apparent descent of the head of the talus in relation to the rest of the foot, which is not associated with any significant increase in the distance between the bony attachments of the plantar calcaneo-navicular ligament. The head of the talus descends towards a given base-line, under the influence of 'passive eversion' of the foot, by an amount which is equivalent to approximately 17 % of the total distance measured, a statistically highly significant difference. The distance between the bony attachments of the 'spring ligament' shows an increased separation in the same position of 2·5 %, which is not significant. Although this evidence is not sufficient in itself it is borne out by the histological evidence. If a purely collagenous ligament is subjected to longitudinal loading it is found that it breaks before it stretches; the ligamentum flavum, on the other hand, which is largely composed of elastic tissue, will stretch under load to approximately 140 % of its unstretched length, and then regain the original length when the load is removed. Histologically the plantar calcaneo-navicular ligament belongs to the former class.

It therefore appears likely that this ligament does not possess elastic properties. It is, however, observed that as the head of the talus descends the apparent long axis of the navicular itself (i.e. the length of its shadow) increases by approximately 10 % of its total length. This apparent change in length is presumed to represent a rotation of the navicular about its short (inter-articular) axis. It is suggested that this rotation is concerned in a movement of the anterior attachment of the plantar calcaneo-navicular ligament laterally and downwards which allows descent of the head of the talus in the circumstances described.

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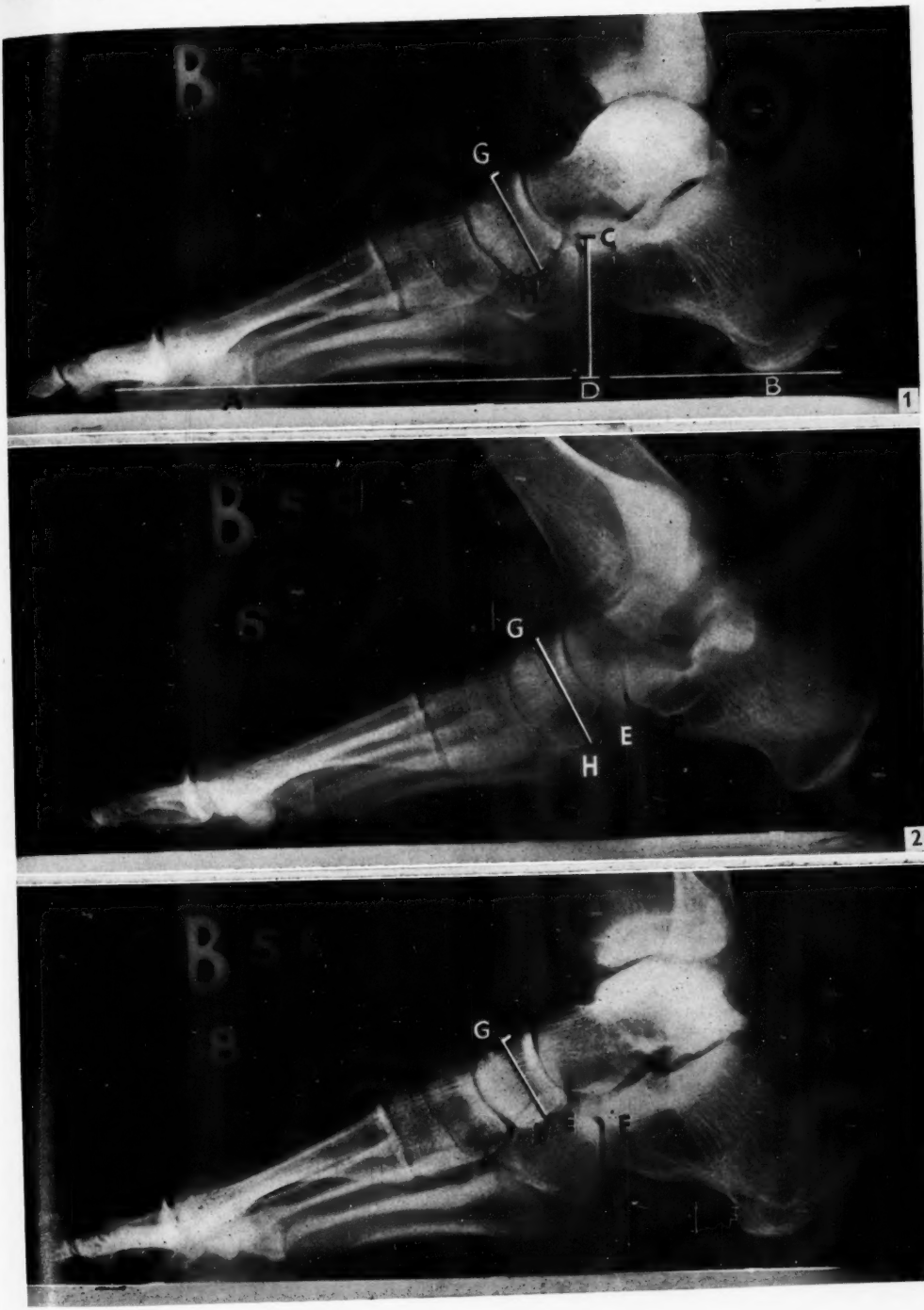
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HARDY—Plantar calcaneo-navicular ligament in man

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EXPLANATION OF PLATES

PLATE 1

Fig. 1. Lateral radiograph of foot in 'standard' position. *AB*, base-line; *CD*, arch height; *GH*, long axis of navicular.

Fig. 2. Lateral radiograph of foot in position of 'passive eversion'. *EF*, bony insertions of 'spring' ligament.

Fig. 3. Lateral radiograph of foot in position of 'active arching' (contraction of tibialis posterior).

PLATE 2

Fig. 4. Human plantar calcaneo-navicular ligament sectioned at 8μ (Ehrlich's haematoxylin). ×30.

Fig. 5. As fig. 4. ×320.

ALKALINE PHOSPHATASE IN THE LARGE INTESTINE

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INTRODUCTION

In contrast to the detailed studies that have been made on the histochemical distribution of alkaline phosphatase in the small intestine, the reports of studies made on the large intestine have been brief and lacking in details.

For the small intestine, it is now firmly established that the enzyme is invariably present at the striated border and, in addition, many of the lining epithelial cells show a discrete supranuclear locus of activity corresponding in position to the Golgi zone (Bourne, 1943; Emmel, 1945).

Since the work of Lundsgaard (1933), it has become fairly generally accepted that a phosphorylation mechanism is involved in the selective absorption of certain monosaccharides, notably glucose. Verzar and his co-workers (see Verzar & McDougall, 1936) claimed to have demonstrated that a phosphorylation mechanism is also operative in the absorption of fatty acids, but a final decision on this point has not yet been reached. Whilst some workers, e.g. Schmidt-Nielsen (1946), support this claim, others, e.g. Barnes, Miller & Burr (1941), Frazer (1946), Zilversmit, Chaikoff & Entenman (1948), do not consider phospholipid formation to be a necessary intermediate stage.

At present, therefore, it seems reasonable to suppose that the alkaline phosphatase at the striated border of the small intestine has some functional relationship to the selective absorption of glucose, but judgement on its relationship to fat absorption must be reserved.

Regarding glucose absorption from the large intestine, opinions amongst physiologists differ a good deal. It seemed therefore of interest to make a detailed comparative study of the histochemical distribution of alkaline phosphatase in the large intestine of various species, in order to determine what relationship there might be between the histochemical and physiological findings. No such comparative histochemical investigation seems to have been made as yet. The data that exist are brief and fragmentary, and in some work it is not even clear to which species the descriptions given relate.

Gomori (1941) has made a short comment on the large intestine of the dog. Having described the distribution of alkaline phosphatase in the small intestine he says, without further elaboration, p. 76: 'In the large intestine the staining is much less intensive but of the same type.'

Kabat & Furth (1941) examined the intestine of adult man, adult chicken, and embryonal mouse. Their report on the large intestine is as follows, p. 306: 'The epithelial cells of the large intestine did not give the reaction, except a few cells in close proximity to the lumen. There were amorphous masses containing phosphatase in the lumen of the gut, and also a few desquamated intestinal epithelial cells which contained only traces of phosphatase. Endothelial cells of the submucosa and of

the lymph follicles almost invariably contained phosphatase and a thin film of phosphatase was found in the serosal layer.'

Bourne (1943), in his study of the guinea-pig, states that the colon showed no reaction in the lining epithelial cells, but that the capillaries were positive. In the rectum, on the other hand, the brush border and distal part of the cytoplasm of the lining epithelial cells gave a reaction.

Deane & Dempsey (1945) found no reaction in the lining epithelial cells of the colon, but the species included in their examination of this region were not given.

Recently, Voge & Bern (1949) studied the cæcum of the red tree mouse (*Phenacomys longicaudus*), and found this species to be unique amongst rodents in possessing well-developed caecal villi (basically similar to those of the small intestine), extending into the first coil of the post-caecal spiral. The entire surface epithelium was intensely positive for alkaline phosphatase activity, particularly in the apical regions of the cells; the reaction continued into the post-caecal spiral, beyond the villus area, but the posterior end of the spiral, like the ascending colon, showed little phosphatase activity.

There are marked species differences in anatomical structure of the large intestine, presumably correlated with differences in physiological function. We may contrast, for example, the large saccular caecum and long thin colon of herbivorous animals, such as the rabbit and guinea-pig, with the small knuckle of a caecum and short wide colon of a carnivorous animal, such as the cat.

The work to be reported here has shown that considerable species variations also occur in the distribution of alkaline phosphatase in the large intestine, but that the pattern of enzyme distribution for each species is remarkably constant.

Since a particular interest lay in the possible presence of enzyme activity at the striated border, special attention was paid to the distribution and degree of development of the striated border in all regions of the large intestines examined.

MATERIALS AND METHODS

The bulk of the material was obtained from six common mammalian species (all normal laboratory animals), and included six rabbits, six guinea-pigs, six rats, six mice, five dogs and eight cats. Human material, available from two relatively recent post-mortem cases, and from two surgical resections, was also investigated.

From each of the laboratory animals, specimens were taken from the caecum, ascending colon, descending colon, and rectum. The human material included sections of descending colon and rectum. All tissues were fixed in 80% alcohol overnight, dehydrated in absolute alcohol, cleared in xylol, and impregnated with paraffin at 56° C. Sections were cut at a standard thickness of 7 μ.

The histochemical technique employed for demonstrating the presence of alkaline phosphatase was a combination of Gomori's (1939) and Kabat & Furth's (1941) techniques, described in full in a previous publication (Martin & Jacoby, 1949). The only substrate employed was sodium-β-glycero-phosphate. No counterstain was used.

Slides prepared from serially mounted sections were labelled from 1 to 5, and treated as follows:

Slide 1. Incubated in the substrate for $\frac{1}{2}$ hr.

Slide 2. Stained with haematoxylin and chromotrop (H. and Chr. section).
Slide 3. Incubated in the substrate for 2 hr.
Slide 4. Incubated in the substrate for 18 hr.
Slide 5. Incubated in the control solution (0·1 % calcium nitrate) for 18 hr.

From each series, a number of sections were superimposed on ready-mounted sections of guinea-pig liver, and the resultant combination subjected to serial incubation periods in the manner previously described (Martin & Jacoby, 1949), in order to determine the time of incubation at which diffusion begins to interfere with the interpretation of results.

There are several advantages to be gained from the above scheme. The H. & Chr. section served not only for the evaluation of the striated border in the various regions of the large intestine, but was also often of assistance in the identification of cells which were found phosphatase positive in the adjacent sections.

The use of several incubation periods provides more information than does a single incubation period. A short incubation period which lies within the time prior to the onset of diffusion demonstrates genuine sites of high enzyme activity, but may be insufficient to bring out sites of weaker inherent enzyme activity. By the use of longer incubation periods—though results are complicated by diffusion—these 'weaker' sites can be evaluated if they are separated from the stronger sites by intervening negative tissue, and if the separate diffusion experiments show that they are outside the range of diffusion. However, localization of all true sites of enzyme activity is scarcely possible.

All control sections were negative, except in the rat, where some of the intestinal contents were almost as brownish black as the contents in the sections which had been incubated with the substrate.

RESULTS

(*A*) *The rabbit*

The large intestine of the rabbit has a large, saccular, spirally wound caecum without taeniae, its distal part being continued into a long and wide appendix. The small intestine opens into the proximal part of the caecum by means of an expansion (the sacculus rotundus), and the colon takes origin from the caecum very close to the entrance of the small intestine.

The proximal portion of the colon, for a distance of 10–20 cm., presents quite a different appearance from the remainder; it possesses three taeniae and well-marked sacculations. The colon beyond this region has one anti-mesenteric taenia for some distance, which then fans out to form a complete investment for the remainder of the colon.

The distribution of alkaline phosphatase

(*a*) *The caecum* (Pl. 1, fig. 1.). After ½ hr. incubation a weak reaction for alkaline phosphatase was obtained at the striated border, but was very incomplete and variable in extent, and in some instances was absent altogether. No other structures gave a reaction at this incubation period.

After the longer incubation periods of 2 and 18 hr., a few small groups of cells in the corium* gave a reaction. A few cells containing a yellow pigment are a feature of the corium, but they did not show a reaction for alkaline phosphatase.

Examination of the sections stained with haematoxylin and chromotrop showed that the striated border is poorly developed.

(*b*) *The proximal colon* (Pl. 1, fig, 2.). In this special region of the colon the mucous membrane forms villus-like projections, some club-shaped and others with pointed tips, each 'villus' possessing many crypts (see also Patzelt, 1936).

After ½ hr. incubation the striated border gave a positive reaction for alkaline phosphatase, stronger than the border reaction in the caecum but a little patchy, sometimes extending for a very short distance into the crypts. The nuclei of the lining epithelial cells were nearly always negative and so was the cell cytoplasm. The corium displayed a very peculiar picture. Patches of small round cells with associated reticular fibres gave a reaction, but these patches of cells and fibres were always confined to the region immediately subjacent to the covering epithelium of the villus-like projections. Lymph nodules usually gave a weak reaction in the peripheral cells. There was no reaction given by the muscle coats or nerve plexuses. Very occasionally capillaries were weakly positive.

After 2 hr. incubation the border reaction was strong but remained patchy, and a diffuse reaction was present in the distal cell cytoplasm. Many nuclei of the lining epithelial cells became positive but, since diffusion from the striated border was shown to be present at this time, their close proximity to this strong site of activity makes evaluation of their activity impossible. Usually some increase in the extent of the corium reaction was observed, more subepithelial patches displaying a reaction. Any lymph nodules that were negative after ½ hr. incubation now showed a reaction in the peripheral cells. After 18 hr. incubation, although diffusion was marked, the outer muscle coats and nerve plexuses remained negative.

In the H. & Chr. sections the striated border was seen to be moderately developed and striations could sometimes be seen extending for a short distance into the crypts, roughly corresponding to the extent of the phosphatase reaction.

(*c*) *The distal colon and rectum* (Pl. 1, figs. 3, 4.). Sections from these two regions of the large intestine, together with some sections from the mid-colon region, gave a similar histochemical picture, so they may be considered together.

After ½ hr. incubation the only reaction obtained was in patches of small round cells in the corium and sometimes in reticular fibres as well (Pl. 1, fig. 5), but these cell patches were not so regionally confined as in the case of the proximal colon. No reaction was given by the lining epithelial cells.

After 2 hr. incubation the corium reaction increased in extent and intensity, but diffusion was becoming evident by this time. The lining epithelium remained negative, apart from a few nuclei of the crypt epithelium, probably as a result of diffusion from the positively reacting structures of the corium.

After 18 hr. incubation there was still no reaction at the intestinal border, nor in the outer muscle coats, nor in the nerve plexuses. The corium reaction had further increased.

* For the sake of brevity, the term corium is used throughout this account when referring to the tunica propria.

Examination of the H. & Chr. sections revealed a very poor development of the striated border, indeed, it was very difficult to identify striations at all.

(*B*) *The guinea-pig*

The guinea-pig has a large, saccular, thin-walled caecum with three taeniae. The small intestine enters the proximal portion of the caecum, and the colon, which is long and thin, leaves the caecum close to the entrance of the small intestine.

The distribution of alkaline phosphatase

(*a*) *The caecum* (Pl. 1, fig. 6). After ½ hr. incubation the striated border gave a very strong reaction in a particular and consistent pattern. The reaction was strongest in the regions adjacent to the openings of the crypts and was continued into the crypts, reaching their extreme depths with little diminution in its intensity. In the surface epithelium between the crypts the border reaction was much weaker and often absent. Both the nuclei and cytoplasm of the lining epithelial cells were mostly negative. A unique feature of the caecum was a positive reaction of the goblet cells, sometimes obtained after ½ hr. incubation but more frequently and strikingly after a longer incubation period. The goblet cells were not very numerous, and were almost entirely confined to the deeper parts of the crypts (Pl. 1, fig. 7); their nature and situation were confirmed by the treatment of suitably fixed specimens with mucicarmine. In no other region of the large intestine of any of the species studied was a goblet cell reaction seen; these cells are well known to be negative in the small intestine. In the corium, reticular fibres together with associated flattened cells gave a strong reaction, and it is noteworthy that, for the most part, the reacting fibres were those closely investing the crypts. Scattered cells also gave a reaction. Capillaries gave a weak, but inconsistent reaction. Pigment-carrying cells, which are a feature of the guinea-pig caecum and more numerous than in the rabbit caecum, were negative. The muscle coats were negative, but Auerbach's plexus sometimes showed a reaction, not in the ganglion cells but in the interganglionic tissue, which gave a granular or very fine reticular appearance in the phosphatase preparations (Pl. 1, fig. 10). The nature of the reacting tissue could not be established, but the possibilities seem to lie between nerve cell processes, Schwann cells, or supporting reticular fibres. Whatever the case may be, a similar appearance was very rarely observed amongst the ganglia of Meissner's plexus. Since there is need to describe this reacting tissue in other regions, the term 'fine network' will be used when referring to it.

After 2 hr. incubation the border reaction, though more intense, maintained its peculiar pattern. Many of the nuclei of the lining epithelial cells became positive but, as in the rabbit, diffusion was evident beyond the ½ hr. incubation period. The cytoplasm presented two interesting features which, in view of the presence of diffusion, must be treated with reserve: (*a*) in a number of cases some of the lining epithelial cells of the crypts showed a supranuclear locus of activity, which appeared as fine threads and granules about mid-way between the nucleus and the striated border; (*b*) the distal cytoplasm displayed a positive granular appearance, merging into the striated border reaction (Pl. 2, fig. 11). It must be emphasized, however,

that the supranuclear locus of activity was uncommon, whilst many cells showed the distal granular reaction. Other cells showed no reaction in the cytoplasm, even after 18 hr. incubation. Silver impregnation of the Golgi element (Aoyama's method) revealed a large reticular network, roughly mid-way between the nucleus and the striated border of the epithelial cells, larger in size and more uniform in appearance than the supranuclear pattern produced by the phosphatase reaction (Pl. 2, fig. 12; compare with fig. 11).

The corium gave a more generalized reaction, but diffusion was much in evidence. The muscle coats remained negative. Any part of Auerbach's plexus which had not shown a reaction in the 'fine network' at the ½ hr. incubation period did so now. The reaction is a genuine one, being set amidst the negative tissue of the muscle coats, and the diffusion experiments showed that diffusion had not reached this locality from elsewhere, even though local diffusion from the 'fine network' often caused an apparent reaction in the ganglion cell nuclei.

After 18 hr. incubation, diffusion was even more marked, making further analysis impossible.

The H. & Chr. sections showed a well-marked striated border which could be followed quite easily down into the crypts, reaching their extreme depths.

(*b*) *The proximal colon, distal colon and rectum* (Pl. 1, figs. 8, 9). Since a similar histochemical picture was obtained in each of these regions of the large intestine, they may be considered together.

After ½ hr. incubation there was usually only slight, variable and patchy reaction at the striated border. The rectum reacted rather the more strongly (see also Bourne, 1943), but even so, some sections showed scarcely any reaction. The nuclei of the lining epithelial cells were nearly always negative, and the cytoplasm was either negative or displayed a fine granular reaction near positive regions of the striated border. The goblet cells were always negative, in contrast to those of the caecum. In the corium, reticular fibres together with associated flattened cells gave a particularly strong reaction, many of them forming a close investment for the crypts. Scattered small cells and some capillaries also gave a reaction. Lymphatic nodules, though sometimes negative at this time, usually showed a weak reaction in their peripheral cells. The muscle coats were negative, but the 'fine network' of Auerbach's plexus sometimes showed a weak reaction.

After 2 hr. incubation the border reaction became a little more extensive, but remained patchy. Many of the nuclei of the lining epithelial cells became positive, especially in the crypts, probably due to diffusion from the strongly reacting corium. The cytoplasm of the epithelial cells remained negative, except in those with a border reaction. The corium showed a more general nuclear reaction, probably from diffusion. Any lymphatic nodules that had not shown a reaction at the ½ hr. incubation period now did so. The muscle coats remained negative. Auerbach's plexus showed a reaction in the 'fine network' and occasionally in the ganglion cell nuclei (from local diffusion); ganglia of Meissner's plexus were consistently negative.

After 18 hr. incubation diffusion was marked, and no further points could be elicited.

In comparison with that of the caecum, the striated border of the colon and rectum, as seen in the H. & Chr. sections, appeared to be poorly developed. Striations could

only be recognized with difficulty in most specimens, and were not seen to extend for more than a very short distance into the crypts.

(*C*) *The rat*

The large intestine of the rat has a fairly large caecum, which is sickle-shaped and usually constricted in several places. The colon, which is smooth-walled, leaves the caecum near the point of entry of the small intestine and forms two simple flexures.

The distribution of alkaline phosphatase

The various regions of the large intestine showed, in the main, a similar histochemical picture and can be considered together. The examination was made on three male and three female animals, and although the males presented a feature that the females did not, further investigations would be needed before one could decide whether there is a true sex difference in this respect.

After ½ hr. incubation, there was normally no reaction obtained at the striated border of any part of the large intestine, except in one animal where a very feeble reaction was seen at the inner border of a few crypts. There was no reaction given by either nuclei or cytoplasm of the lining epithelial cells. The corium showed a reaction in scattered cells, many of which seemed to correspond in size and distribution to eosinophils seen in the neighbouring H. & Chr. sections. The male animals showed, in addition, a reaction in reticular fibres and associated flattened cells. The muscle coats in the case of the female animals were negative apart from scattered positive cells and capillaries. In the male animals, a striking picture was obtained; the reticular fibres of the inner muscle coat were positive, whilst the outer muscle coat was negative (Pl. 2, figs. 13, 14). Auerbach's plexus showed a reaction in the 'fine network' in some of the animals. The vascular reaction pattern consisted of a positive reaction in capillaries and in the adventitia of the arteries, whilst the veins were negative.

There was a progressive decrease in the reaction given by all structures of the distal segments of the large intestine (Pl. 2, figs. 13 and 15–17).

After 2 hr. incubation, diffusion from the positive corium tissue was occurring, but there was still no reaction given by the border of the lining epithelial cells. The corium showed a more wide-spread nuclear reaction, and often more reticular fibres had become positive. With the distal colon and rectum, this longer incubation period was necessary to demonstrate a reaction in the reticular fibres.

After 18 hr. incubation some nuclei of the lining epithelial cells became positive, mostly those of the crypts, and obviously due to diffusion from the positive corium tissue. The cell cytoplasm and striated border remained negative.

Although it was difficult to identify striations in most sections of the large intestine stained with H. & Chr., a low but clearly represented striated border was met with occasionally, but it was not seen in the crypts.

(*D*) *The mouse*

The large intestine of the mouse has a small flattened caecum, which receives the small intestine on its left side. From the apex of the caecum a rapidly narrowing appendix descends towards the pelvis, and from the opposite end the smooth-

walled colon takes origin from the caecum, forming two simple flexures in its course.

The distribution of alkaline phosphatase

The proximal segments of the large intestine gave a histochemical picture different from that of the distal parts.

(*a*) *The caecum* (Pl. 2, fig. 18). After ½ hr. incubation the striated border gave a strong but slightly patchy reaction, extending for a fair distance into the crypts, and although it increased in intensity after the longer incubation periods, it was unchanged in extent. Most of the nuclei of the lining epithelial cells were negative; those that gave a reaction were in regions of a strong border reaction. No cytoplasmic reaction was seen. In the corium small groups of cells, for the most part near the bases of the crypts, were weakly positive. The muscle coats and nerve plexuses were negative. The vascular reaction pattern consisted of a weak capillary reaction and a variable arterial reaction (sometimes in the endothelium, sometimes in the adventitia), whilst the veins were negative.

Beyond the ½ hr. incubation period diffusion was apparent, but it is likely that the reticular fibre reaction which usually became evident in the corium after the longer incubation periods is genuine, and not a diffusion artefact.

Examination of the H. & Chr. sections revealed a well-developed but patchy striated border, easily traced for some distance into the crypts, but not into their deeper parts.

(*b*) *The proximal colon* (Pl. 3, fig. 19). The results obtained for this segment were in all respects similar to those in the caecum, but the reaction at the striated border was somewhat weaker and more patchy. The striated border itself, as seen in the H. & Chr. sections, appeared less well developed than in the caecum and extended for a shorter distance into the crypts.

(*c*) *The distal colon and rectum*. The histochemical findings in these parts of the intestine were identical (Pl. 3, figs. 20, 21).

After ½ hr. incubation there was never any reaction given by the lining epithelial cells, and the striated border remained negative even after 18 hr. incubation. The corium, too, was in most instances entirely negative, but a few specimens showed a weak reaction in small groups of cells. A recticular fibre reaction was not observed. Lymph nodules sometimes showed a weak reaction in the peripheral cells. The muscle coats and nerve plexuses were negative.

The striated border, as seen in the H. & Chr. sections, was very poorly developed in these regions.

(*E*) *The dog*

The large intestine of the dog has a very short caecum, and a relatively short wide colon, which forms two ill-defined flexures.

The distribution of alkaline phosphatase

All regions of the large intestine showed an identical distribution of alkaline phosphatase (Pl. 3, fig. 22), so that the following account applies to all its parts.

After ½ hr. incubation a strong reaction was given by the striated border, passing into the crypts about half way down (occasionally a little deeper) and gradually

fading, so that no reaction was seen in their deeper parts. After prolonged incubation no change in the extent of the reaction occurred. The nuclei of the lining epithelial cells were usually negative, but their cytoplasm showed, in addition to a diffuse reaction adjacent to the striated border, a discrete supranuclear locus of activity situated nearer to the nucleus than to the striated border (Pl. 3, fig. 23). The corium presented a unique appearance in the phosphatase preparations. There was invariably a reaction in a narrow band of small round cells and associated reticular fibres lying between the bases of the crypts and the muscularis mucosae. Gomori (1941) described a positively reacting band of tissue in the corresponding region of the small intestine, which he referred to as connective tissue. Moll (1888) is quoted by Patzelt (1936) as having described this layer in the dog's intestine under the name of 'stratum granulosum'. Patzelt's account includes later reports on this zone, stating that it is much narrower in the large than in the small intestine of the dog; that it is composed of numerous lymphocytes and fine reticular fibres, and that it probably represents a zone of flattened lymphoid follicles.

In pursuing this matter, I tested sections of dog's duodenum for alkaline phosphatase, and found that this zone of small round cells gave a strong reaction and was, indeed, wider than in the colon. It seems, therefore, that the reacting zone corresponds to the 'stratum granulosum' of Moll, and with regard to the interpretation of this zone, it is noteworthy that the peripheral cells of solitary lymph nodules give a reaction for alkaline phosphatase.

Apart from the 'stratum granulosum', there was little else displaying a reaction in the corium, only here and there small groups of small round cells between the crypts and occasionally reticular fibres investing the bases of the crypts. Lymphatic nodules often showed a reaction in the peripheral cells and associated reticular fibres. There was seldom a reaction given by the capillaries. The muscle coats and nerve plexuses were invariably negative, and remained so even after 18 hr. incubation.

In the H. & Chr. sections, the striated border of the surface epithelium throughout the large intestine was seen to be well developed, and could be traced into the crypts for a considerable distance, but became very indistinct in their deepest parts.

(*F*) *The cat*

The large intestine of the cat is typical of the carnivorous animals. The caecum is reduced to a small conical knuckle without any appendix and the colon is very short, passing slightly obliquely across the abdomen, to end in the rectum without forming flexures.

The distribution of alkaline phosphatase

Of all the species examined, the cat exhibited the most striking reaction for alkaline phosphatase in the large intestine. Sections taken from all levels of this part of the gut gave an identical histochemical picture, without any apparent regional differences in the intensity of the reaction (see Pl. 3, figs. 24, 25).

In attempting to localize the sites of genuine enzyme activity in this species, the use of a series of incubation periods was particularly necessary. Diffusion was found to occur to a considerable extent after $\frac{1}{2}$ hr. incubation, so that even shorter

incubation periods were needed for study. As had been the case with other species however, some apparently genuine sites of activity did not always manifest themselves until incubation periods of more than ½ hr. were employed.

The histochemical picture obtained after a 15 min. incubation period was usually uncomplicated by diffusion. At that time, a complete and very intense reaction was present at the striated border, passing into the crypts to their extreme depths and diminishing only a little in intensity in their deeper parts. The contents of the crypts were also strongly positive. Although the nuclei of the lining epithelial cells were negative at this period, the distal cytoplasm of the cells, particularly in the crypts, gave a diffuse reaction. The corium showed a reaction in reticular fibres and associated flattened cells investing the bases of the crypts. All else was negative.

Because of the intensity of the reaction at the striated border, it was of interest to test the reaction after still shorter incubation periods. It was found that a reaction was given by the striated border of both the surface and the crypt epithelium after an incubation period of 2 min., but was not quite complete over the whole border. Even after this short incubation, a reaction was obvious in the distal part of the cytoplasm of the lining epithelial cells of the crypts, yet no reaction was given by other structures. Examination of the cytoplasmic reaction with an oil-immersion lens revealed the presence of phosphatase-positive granules, the larger ones situated just beneath the striated border and other smaller ones scattered in the cytoplasm (Pl. 3, fig. 26). A distinct supranuclear focus of activity was not observed.

An incubation period of ½ hr. (sometimes longer) brought out a reaction in the peripheral cells of lymph nodules, in the 'fine network' of Auerbach's plexus, and occasionally in a similar network of Meissner's plexus. Although diffusion was present, it could be shown that these regions were genuine sites of enzyme activity.

As seen in the H. &. Chr. sections the striated border was particularly well developed in all regions of the large intestine, and could be traced to the depths of the crypts, but became less well marked in their deeper parts.

(*G*) *Man*

The large intestine of man is structurally intermediate between the herbivorous and carnivorous types, i.e. of the omnivorous pattern.

The distribution of alkaline phosphatase

The human material studied included rectum and distal portions of the colon only. It was not possible to obtain operation material from the caecum or proximal parts of the colon, and the post-mortem material from these regions was not sufficiently well preserved for study.

After ½ hr. incubation, a very feeble reaction for alkaline phosphatase was obtained at the striated border in some of the specimens; an incubation for 2 hr. was often needed to bring out a reaction at all; it increased slightly after 18 hr. The reaction was always weak and in small patches, but extended into the crypts to about one-third of their length. The lining epithelial cells showed no reaction in nuclei or cytoplasm after ½ hr. incubation. The corium showed a striking reaction in the capillaries, but all else was negative, except for an occasional weak reaction in lymph nodules. The muscle coats and nerve plexuses were negative and remained

so after prolonged incubation. Pl. 3, fig. 27, shows the strongest border reaction that was obtained in any specimen after a 2 hr. incubation period.

As seen in the H. & Chr. sections the striated border was fairly well developed, and could be traced into the crypts for some distance before becoming indistinct.

Table 1. *The histochemical reaction for alkaline phosphatase at the striated border, and in Auerbach's plexus of the large intestine*

		Border reaction		Reaction in Auerbach's plexus
		Surface epithelium	Crypts	
Cat	Caecum	+ + +	+ + +	+
	Proximal colon	+ + +	+ + +	+
	Distal colon	+ + +	+ + +	+
	Rectum	+ + +	+ + +	+
Dog	Caecum	+ +	+ + ($\frac{1}{3}$)	−
	Proximal colon	+ +	+ + ($\frac{1}{3}$)	−
	Distal colon	+ +	+ + ($\frac{1}{3}$)	−
	Rectum	+ +	+ + ($\frac{1}{3}$)	−
Guinea-pig	Caecum	+ + P.	+ +	+
	Proximal colon	(±) P.	−	+
	Distal colon	(±) P.	−	+
	Rectum	(±) P.	−	+
Mouse	Caecum	+(+) P.	+(+) ($\frac{1}{3}$)	−
	Proximal colon	+P.	+(<$\frac{1}{3}$)	−
	Distal colon	−	−	−
	Rectum	−	−	−
Rabbit	Caecum	(±) P.	−	−
	Proximal colon	+ P.	−	−
	Distal colon	−	−	−
	Rectum	−	−	−
Rat	Caecum	−	−	+
	Proximal colon	−	−	+
	Distal colon	−	−	(+)
	Rectum	−	−	(+)
Man	Distal colon	(+) P.	(+) ($\frac{1}{3}$)	−
	Rectum	(+) P.	(+) ($\frac{1}{3}$)	−

+ + +, + +, +(+), +, (+) = relative strength of the reaction.
(±) = weak reaction, or none.
− = no reaction.
P. = patchy reaction.
Fractions = depth to which reaction passes into crypts.

DISCUSSION

The present study provides yet another example of the marked species variations which exist in the histochemical distribution of alkaline phosphatase at certain sites. In order to give a synoptic view of the more important differences in enzyme distribution met with in the present investigation, Table 1 is provided.

Unless we take the rather passive attitude that these differences are merely phylogenetic freaks, with no significance, we have to assume that these species variations reflect differences in physiological behaviour; and such comparative histochemical studies might therefore be helpful in the elucidation of physiological problems.

The most important feature brought out in this investigation is the varying behaviour of the alkaline phosphatase reaction at the striated border on the surface

and in the crypts. The *cat* is foremost in this respect, giving a heavy reaction in all parts of the large intestine; the *dog* is a close second. Next comes the *guinea-pig*, with a very definite reaction in the caecum, especially in the crypts, whilst in the rest of the large intestine the reaction fades away; the *mouse* is somewhat similar in behaviour, except that the distal colon and rectum are always found to be completely negative. In the *rabbit*, it is principally the proximal colon (on the peculiar anatomical structure of which we have already commented) which gives a more or less consistent reaction at the striated border. Lastly there is the *rat*, in which the striated border was found consistently negative over the whole length of the large intestine. The *human* material, being incomplete and not very well preserved, has to be considered separately, but it does most closely resemble the tissue in the dog, though reacting much less strongly.

If one assumes that the presence of the enzyme at the striated border has a functional relationship to glucose absorption, as is generally thought to be the case in the small intestine, there should be a correspondence between the histochemical findings and available physiological data for the large intestine, and if such correspondence were found to exist, histochemical results might prove a useful indicator of absorptive capacity in species that have not as yet been subject to physiological study. The degree and extent of the striated border reaction might also provide some guide to the amount of glucose absorption normally taking place in an intestinal segment.

One would expect, if these assumptions are correct, that a considerable absorption of glucose takes place throughout the large intestine of the cat, and to a somewhat less extent throughout the large intestine of the dog. Man might be expected to absorb glucose to a slight extent.

In direct contrast to this group, the rat would not be expected to absorb glucose from any part of the large intestine. All the other species studied should exhibit segmental differences in absorptive capacity, viz. marked absorption from the caecum of the guinea-pig and very much less from the colon; absorption from about the proximal half of the large intestine of the mouse and none from the distal half, and in the rabbit, absorption from the specialized proximal region of the colon (perhaps also a little from the caecum), and none from the remainder of the large intestine.

Unfortunately, complete physiological reports are not available for all these species, and there is not always agreement in the existing data.

The cat was investigated by Büdingen (1935), who found that glucose was readily absorbed from closed loops of the colon if the solutions were slightly hypertonic, but that much less absorption took place of other concentrations and none of an isotonic solution.

The dog has been fairly extensively studied. The earlier investigations (reviewed by Carpenter, 1925) indicated that glucose is readily absorbed from the dog's large intestine, but this has not been supported by more recent work. McNealy & Willems (1929, 1931) came to the conclusion that glucose is not absorbed unless instilled with normal saline, and then only to a slight extent. Ebeling (1933) found a slight absorption in the normal and in the hyperglycaemic dog, and marked absorption in the hypoglycaemic animal. Burget, Moore & Lloyd (1933), using chronic closed loops of colon, claimed that a ready absorption could be shown.

In the light of these reports, it is probably fair to assume that the dog is able to absorb glucose from the large intestine, but under experimental conditions the amount does not seem to be large.

Studies on man have given similar results to those on the dog. Earlier work (reviewed by Carpenter, 1925) indicated that glucose is fairly readily absorbed from the large intestine, and Carpenter himself found that glucose is absorbed from a glucose-saline enema. More recently, Pressman (1930), and Scott & Zweighaft (1932) concluded from studies made by rectal instillation of glucose that glucose is absorbed only to a slight extent. Curry & Bargen (1935) studied glucose absorption from isolated colon segments in colostomized patients and also came to the conclusion that only small amounts of glucose are absorbed. A different method of approach to the problem was used by Welch, Wakefield & Adams (1936), who compared analyses of the contents of the ileum with those of normal faeces on a patient who had undergone an operation of ileostomy, with the colon left *in situ*. They found that there was an excess of water and electrolytes, but not of protein, fat, or carbohydrate in the dejecta from the ileum, when compared with faeces. The inference drawn was that the large intestine is normally called upon to absorb water and electrolytes, but not the other substances.

The experimental results suggest, in the main, that no more than a slight absorption of glucose takes place from the large intestine of man.

The rat was first studied by Davidson & Garry (1939), who found no evidence of glucose absorption from any part of the large intestine. More recently, Navarro (1946) found very little absorption of glucose from the large intestine of the rat, and suggested that simple diffusion would perhaps account for the small quantity absorbed.

It is of interest that he found no secretion of alkaline phosphatase by the rat's colon, and that the wall of the colon was poor in phosphatase content on chemical analysis—observations which accord well with the histochemical findings.

The guinea-pig was also studied in some detail by Navarro (1946). He found that although glucose was absorbed from the colon, the amount was very much less than from the small intestine, the absorption ratios from the jejunum, terminal ileum, and colon being 1·0, 0·7, and 0·3 respectively. There was either no secretion of alkaline phosphatase from the colon or only very small amounts, and the wall of the colon contained very little phosphatase on chemical examination. He found that the wall of the caecum did not have a very high content of phosphatase, but the caecal contents had, giving on occasion even a higher value than those of the small intestine; a circumstance which he attributed to absorption of water from the caecal contents with resulting concentration of the contained phosphatase. Glucose absorption from the caecum was not studied.

For the rabbit and mouse, there does not seem to be any information available on glucose absorption from the large intestine, but Larson & Bargen (1933), in their review of the physiology of the colon, quote Maestrini as having found in the rabbit, by means of colon stomata, that absorption of nitrogenous substances and fats occurred from the *proximal* part of the colon only, i.e. from that region of the colon from which glucose absorption, judged from the histochemical findings, would be expected to occur.

It will be seen therefore, that, as far as they go, the results of physiological studies are in some respects in fair agreement with the deductions made from the histochemical findings. However, caution needs to be exercised in reaching a final verdict on the relationship, since the existing physiological information is incomplete and sometimes contradictory. Interest would lie particularly in the results of absorption studies on the mouse's large intestine, in further work on the guinea-pig (to include the caecum), and on the rabbit. From the histochemical findings, it might be expected that segmental differences in absorptive capacity would be found in all these species.

The next important point that has to be considered is the relationship of the reaction for alkaline phosphatase to the extent to which the striated border is developed. The latter point in each case has been determined by an examination of sections stained with haematoxylin and chromotrop after 80% alcohol fixation. It was observed that the enzyme is not found in regions where a striated border is not obvious. On the other hand, it must be admitted that a striated border does not necessarily have alkaline phosphatase associated with it. This was to be seen particularly in the rat; the large intestine has a striated border (not very high, but often clearly represented), yet no reaction for alkaline phosphatase was found in association with it.

With regard to a positive border reaction, examination of the crypts yielded particularly interesting information. It was found that the striated border, as seen in H. & Chr. sections, usually became indistinct as their deeper parts were approached, the level at which it faded depending on the species. The reaction for alkaline phosphatase faded at much the same level in the crypts as did the striated border. In some regions, the reaction for alkaline phosphatase was very intense, and extended to the deepest parts of the crypts. This was the case in the guinea-pig caecum and throughout the large intestine of the cat. In these regions, it was usually possible to trace the striated border also to the deepest parts of the crypts.

The presence of a striated border with associated alkaline phosphatase in crypts is very puzzling. Does it indicate that the crypts of the large intestine in some species are absorptive in function? The crypts are commonly regarded as secretory and not absorptive structures, and indeed would not appear to be mechanically well adapted for an absorptive role. It is possible, however, that the crypts open out during peristalsis, so that their lining epithelial cells are brought into more direct contact with the intestinal contents.

The presence of a striated border very deep in the crypts in itself raises the possibility that absorption (even if only of water and electrolytes) takes place from these structures. Although the available evidence suggests that the associated enzyme is related to glucose absorption, we must keep an open mind on the matter until further data become available, and meanwhile concede the possibility that the enzyme in the large intestine may have a more limited functional role, or even a different one from that which has been suggested for the small intestine. The enzyme at the striated border may play some part in the excretion of certain substances; phosphates obviously come to mind. Again, it is possible that the enzyme concentrated at the striated border is merely undergoing secretion into the intestinal contents, and does not play any part in chemical absorption processes,

whereas in the small intestine it is likely that the enzyme at the border has a functional role (i.e. absorption of foodstuffs) different from that of the enzyme secreted into the contents. Prior to the work of Navarro (already referred to) on the guinea-pig and rat, Kosman, Kaulbersz & Freeman (1943) showed that alkaline phosphatase is secreted by the dog's intestinal mucosa, the colon giving the lowest values, and they suggested that the secreted enzyme may be concerned with hydrolysis of ingested monophosphoric esters.

It is well known that in histochemically treated sections of the small intestine a strong reaction is given by the intestinal contents. In the present study, it was observed that the contents of the large intestine were strongly positive only in regions that gave a reaction at the striated border; in regions where a border reaction was absent, the contents reacted weakly or not at all. The inference might be drawn that the enzyme is largely destroyed in its passage through the intestine (Navarro (1946) reached the same conclusion from chemical analysis), and that if large quantities are needed in the lumen of the large intestine the provision is made by the large intestine itself. The high concentration of the enzyme in the contents of the large intestine of certain species may be related to a high proportion of monophosphoric esters entering the large intestine from the small. A comparative chemical study of the normal contents on entering and on leaving the large intestine might prove to be a step towards the solution of the problem. Because of the marked contrast in the histochemical findings which they present, the cat and the rat would seem to be species suitable for such comparative work.

In addition to a reaction at the striated border, intracellular activity in the lining epithelial cells was often present. It is open to question whether their nuclei contain histochemically demonstrable alkaline phosphatase, but they seem to be negative prior to the onset of diffusion, and in specimens which show no border reaction, they remain negative after prolonged incubation, unless reached by diffusion from positive corium tissue. Intracytoplasmic activity was commonly seen in cells showing a border reaction, but was absent from those without this feature. In some cases, a discrete supranuclear focus of activity was seen prior to the onset of diffusion, being particularly noticeable in the epithelium lining the crypts of the dog's large intestine. In general, a granular reaction was more common, and for the most part confined to the distal part of the cell. In the cat's large intestine, short incubation periods demonstrated such positive granules very clearly, the larger ones situated near to the striated border. It is tempting to think of the granules as cell products destined for the striated border, and perhaps eventually for secretion into the intestinal lumen, but alternatively they may be associated with intracellular metabolism. It is not possible to say whether they are elaborated in the Golgi zone, nor whether the activity sometimes seen in the Golgi zone is associated with intracellular metabolism. The diffuse cytoplasmic reaction seen on prolonging the incubation period, in cells which showed positive granules earlier, is in all probability due to local 'internal' diffusion from these granules.

The reaction in the connective tissue showed species variations, and may be considered under the headings of vascular, cellular, and fibrous.

In accord with the results of other workers, it was found that a blood-vessel reaction was nearly always confined to the endothelium of capillaries, but in some

species it was feeble and irregular, e.g. cat, dog and rabbit. In the case of the rat, and occasionally in the mouse also, arteries frequently showed an adventitial reaction. It is interesting in this connexion to refer to the observation of MacFarlane, Patterson & Robison (1934), that the aorta of the rat differs from that of other species in giving a positive reaction for alkaline phosphatase on chemical examination.

The reaction given by the reticular fibres of the corium, though puzzling as to its significance, needs some special comments. In some cases (e.g. man) it is not evident, in others (e.g. guinea-pig, dog and cat) it is seen especially around the crypts or crypt bases, whilst in others (e.g. rat) it shows a more 'open' arrangement. There is also a good deal of individual variation in the intensity of the reaction.

A fibre, or stromal reaction, has of course been described in many tissues, but its significance is quite obscure. It might suggest that dephosphorylation occurs in the tissue fluid; dispersion of the enzyme on the fibrous stroma would provide a suitably arranged mechanism for the purpose.

With regard to the cellular reaction in the corium, it is difficult to decide how much of it is due to diffusion, particularly when there is a strong reticular fibre or capillary reaction, and a detailed study was not attempted. The rather weak reaction given by the peripheral cells of lymphatic nodules, however, seems to represent a genuine site of activity.

The nuclei of smooth muscle fibres can be regarded as negative, any reaction that they show becoming evident only after diffusion from other sites has reached them.

The reaction ('fine network') seen in the ganglia of Auerbach's plexus (rarely in Meissner's) in several of the species examined (guinea-pig, rat and cat) almost certainly represents genuine enzyme activity. As previously said, the decision upon the nature of this reacting tissue lies between nerve cell processes, Schwann cells, or just reticular fibres. Of interest in this connexion are the observations of Gomori and of Marchant. Gomori (1941) states: 'in sympathetic ganglia the satellite cells and some of the connective tissue separating the ganglion cells have been found positive, although not quite uniformly so in all species'. Marchant (1949), in an investigation on the rabbit, noted that the tissue surrounding the ganglion cells of an autonomic ganglion gave a very intense reaction. She also described the presence of phosphatase-positive streaks in normal sciatic and in several autonomic nerves, and although the evidence was not regarded as conclusive, it was suggested that the streaks represented unmyelinated fibres and their associated Schwann cells.

Further investigation is obviously needed to determine the nature of the tissue which gives this reaction.

SUMMARY

1. A brief description of the gross anatomy of the large intestine of seven species is given.

2. A comparative histochemical study of the distribution of alkaline phosphatase in the large intestine of these species has been made, together with an examination of the striated border.

3. Marked species variations in the distribution of the enzyme were encountered,

and in some species marked differences in enzyme distribution at different levels of the large intestine.

4. A relationship was found between the development of the striated border and the presence of alkaline phosphatase at the border.

5. The significance of the findings in relation to physiological studies on absorption from the large intestine is discussed, and as a result of the study, the suggestion is put forward that the crypts of the large intestine of certain species may play a role in the absorption of foodstuffs.

I wish to express my thanks to Dr F. Jacoby for his helpful criticism and advice throughout this work, to Mr A. Welch for his assistance in taking the microphotographs, and to Mr L. Jones for his help in the cutting of sections.

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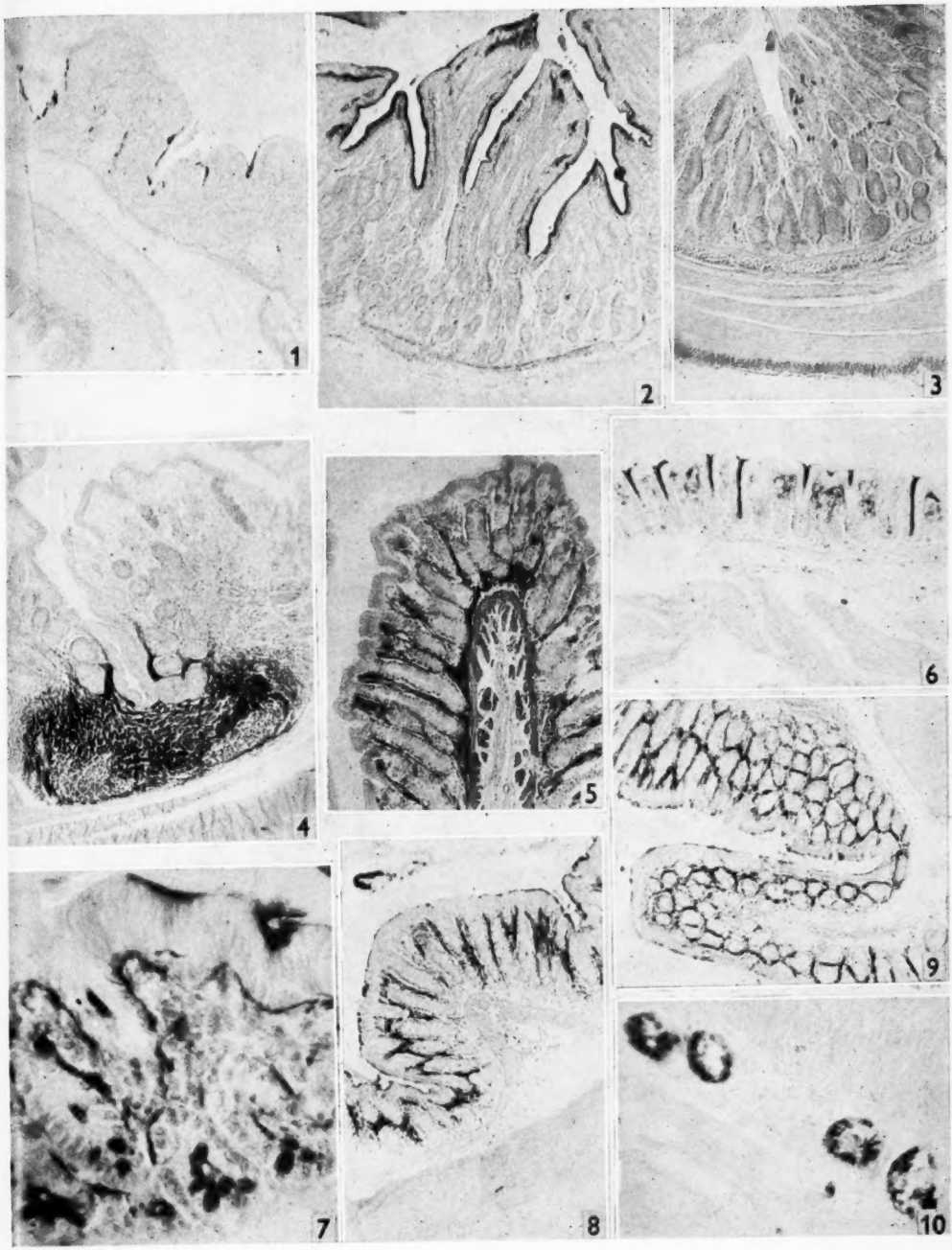
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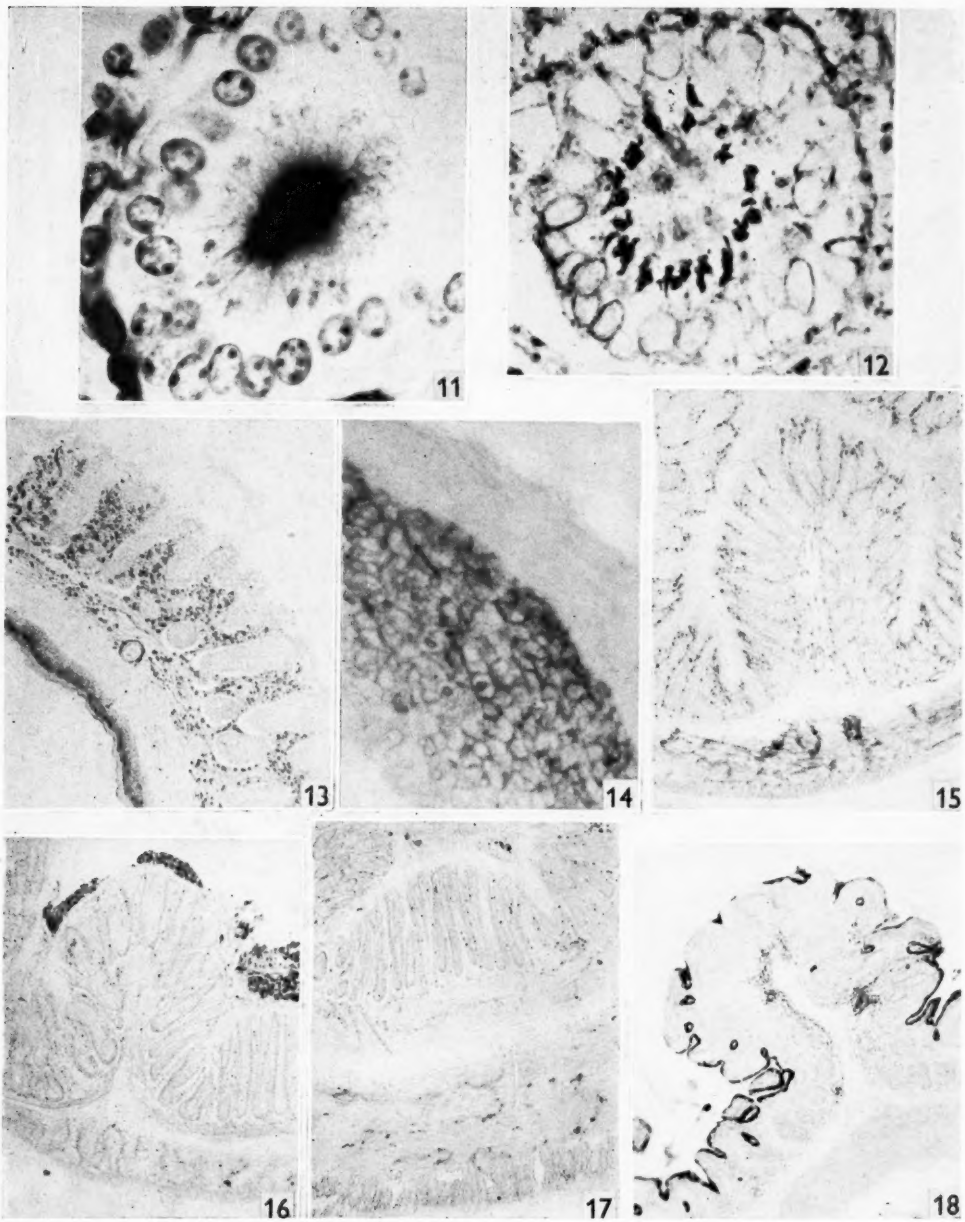
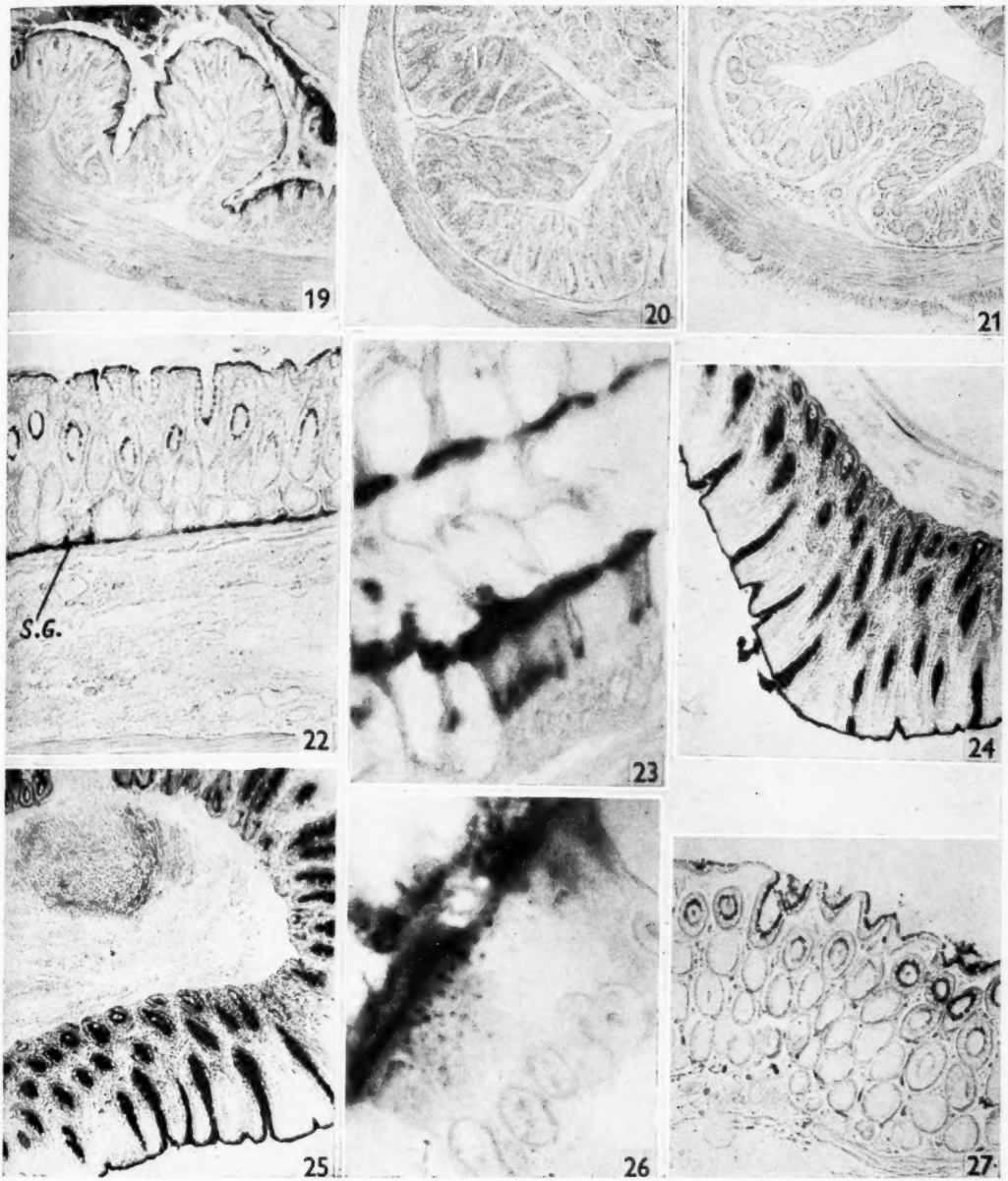


MARTIN—ALKALINE PHOSPHATASE IN THE LARGE INTESTINE



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EXPLANATION OF PLATES

All photographs were taken through a Wratten G filter.

PLATE 1

Fig. 1. Rabbit, caecum. ½ hr. incubation. A weak, patchy reaction at the striated border is the only reaction. × 65.

Fig. 2. Rabbit, proximal colon. ½ hr. incubation. Note villus-like processes. A moderate, but slightly patchy reaction at the striated border. A subepithelial reaction is seen in the corium. × 65.

Fig. 3. Rabbit, distal colon. ½ hr. incubation. There is only a reaction in a few cells of the corium. × 65.

Fig. 4. Rabbit, rectum. ½ hr. incubation. A lymph nodule shows a reaction in the peripheral cells; all else is negative. × 65.

Fig. 5. Rabbit, distal colon. ½ hr. incubation. In this specimen, a strong reticular fibre reaction is seen. × 65.

Fig. 6. Guinea-pig, caecum. ½ hr. incubation. A strong reaction is seen at the striated border of the crypt epithelium and adjacent parts of the surface epithelium. A strong reaction is seen in the corium. × 65.

Fig. 7. Guinea-pig, caecum. 2 hr. incubation. Higher power of another field to show positive goblet cells. × 300.

Fig. 8. Guinea-pig, distal colon. ½ hr. incubation. Feeble, patchy reaction at the striated border, and a strong corium reaction. × 65.

Fig. 9. Guinea-pig, rectum. ½ hr. incubation. Similar picture to distal colon (fig. 8). × 65.

Fig. 10. Guinea-pig, caecum. 2 hr. incubation. Field showing a reaction in the 'fine network' of ganglia of Auerbach's plexus. × 210.

PLATE 2

Fig. 11. Guinea-pig, caecum. 2 hr. incubation. A crypt in transverse section showing supranuclear activity and a distal granular reaction in the lining epithelial cells. × 890.

Fig. 12. Guinea-pig, caecum. Silver impregnation to show the Golgi apparatus in the crypt epithelium. Compare with fig. 11. × 890.

Fig. 13. Rat, caecum. ½ hr. incubation. Reaction in scattered cells, reticular fibres and capillaries. Note adventitial reaction in an artery. Strong reaction in reticular fibres of inner muscle coat. × 65.

Fig. 14. Rat, caecum. ½ hr. incubation. Higher power to show reaction in inner muscle coat. × 625.

Fig. 15. Rat, proximal colon. ½ hr. incubation. Similar to caecum, but reaction less intense. × 40.

Figs. 16, 17. Rat, distal colon and rectum respectively. ½ hr. incubation. Further decrease in the reaction. The intestinal contents in the control sections appeared almost equally dark. × 40.

Fig. 18. Mouse, caecum. ½ hr. incubation. Strong, slightly patchy reaction at striated border, passing for a fair distance into the crypts. Weak reaction in the corium. × 65.

PLATE 3

Fig. 19. Mouse, proximal colon. ½ hr. incubation. Similar to caecum, but reaction less intense. × 65.

Figs. 20, 21. Mouse, distal colon and rectum respectively. ½ hr. incubation. No reaction except in a few cells of the corium. × 65.

Fig. 22. Dog, rectum. ½ hr. incubation. Strong reaction at striated border, passing for a considerable distance into the crypts. Note strong reaction in the 'stratum granulosum' (s.g.). × 65. A similar picture is seen in all parts of the dog's large intestine.

Fig. 23. Dog, colon (crypt). ½ hr. incubation. Reaction in Golgi zone of lining epithelial cells. × 890.

Fig. 24. Cat, caecum. ½ hr. incubation. Intense reaction at striated border, passing to extreme depths of the crypts. Some reticular fibre reaction around the crypt bases. × 65.

Fig. 25. Cat, rectum. ½ hr. incubation. Essentially the same picture as in the caecum. Note reaction in the peripheral cells of a lymph nodule. × 65.

Fig. 26. Cat, caecum (crypt). 2 min. incubation. A positive reaction is seen in granules, mostly located just beneath the striated border. × 1,300.

Fig. 27. Man, rectum. 2 hr. incubation. Patchy weak reaction at the striated border, passing for some distance into the crypts. Capillary reaction is conspicuous. × 43.

POSTNATAL CHANGES IN THE LOBULATION AND CONNECTIVE TISSUE OF THE PANCREAS

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The histological appearance of many tissues in the newborn child differs from that in the adult. When discussing disorders of the pancreas, many pathologists who are experienced in the morbid anatomy of childhood make casual reference to the difference between the adult and infantile gland (e.g. Potter & Adair, 1949, Baar, 1946). Anderson (1938), in her well-known article on 'Cystic fibrosis in the pancreas and its relation to celiac disease', was careful to show photographs of the normal pancreas in children of the same age as those showing lesions. Popular text-books of histology (Maximow & Bloom, 1947, Schafer, 1950), make no reference to the pancreas in children, and von Möllendorff (1927), who described the connective tissue of the pancreas with great accuracy and detail, does not refer to differences between the infantile and adult gland. Cowdry (1942), when discussing ageing of the pancreas, infers that the same post-natal changes occur as in the salivary glands. He quotes the finding of Scammon that in the salivary glands the connective tissue and ducts are more plentiful in the infant and that the glands do not show the adult histological picture until the end of the second year. Instances could be cited, e.g. Riniker (1946), where photomicrographs of an infant's pancreas are printed and described as abnormal when the organ is probably normal for the age of the child.

In assessing pathological changes in an organ it is necessary to have a clear picture of the normal variation which can be expected at different age levels. Two studies of the pancreas concerned with the post-natal changes in the interstitial connective tissue have recently been published. Kitaygorodskaya (1944) examined the pancreas from 102 persons of all ages coming to medico-legal autopsy in Moscow. The cases were divided into six groups: (1) foetal; (2) birth to $1\frac{1}{2}$ years ('period of toothless childhood'); (3) $1\frac{1}{2}$–7 years ('milk dentition'); (4) 7–11 years; (5) 11–16 years ('adolescent'); (6) adult. It was noticed that with increasing age there was a gradual diminution in the amount of connective tissue, the adult type of gland not being found until the age of 11–16 years. The appearances of the gland at different ages were not illustrated, and no data were given against which any gland could be assessed. Ule (1948) examined the pancreas in seventy-two infants and found a high incidence of lesions affecting the connective tissue. He suggested that in the first year of life the connective tissue of the pancreas serves some unknown physiological function.

The purpose of the present study has been to ascertain the normal variation to be expected in the 'low-power' microscopical appearance of the pancreas at different ages in childhood.

MATERIAL

Blocks of the head and terminal third of the pancreas have been taken from all children coming to autopsy at the Sheffield Children's Hospital since 1947. On examination of all sections, all cases were excluded which showed any pathological

lesion of the pancreas, e.g. 'cystic fibrosis', tumour infiltration or haemorrhage, or in which there had been marked post-mortem autolysis. Small collections of lymphocytes in the connective tissue were considered normal and did not entail exclusion of a case. The number of cases remaining, and later analysed, was 200.

METHOD

The histological appearance of the pancreas was divided into six categories; the first representing the pancreas found in the still-born or premature child, and category six the normal adult pancreas. The grading was based on the following points, which are all aspects of the same general change in the features of the gland:

(*a*) The relative amount of connective tissue to parenchymal tissue present.

(*b*) The size of the connective tissue bands dividing the lobes and lobules.

(*c*) The number of acini in a lobule, i.e. the size of the lobule.

(*d*) The shape of the lobules.

Characteristic appearances of the various categories are illustrated in Pls. 1 and 2.

In category 1 the acini are widely separated and a large number are single. Connective tissue constitutes as much of the section as does the parenchymal tissue. In category 2, a few isolated acini are still present, but most are grouped in rounded masses. These small lobules lie relatively free and are little moulded by surrounding lobules.

In category 3 the lobules are more prominent and in some areas are packed together, but the majority are still free and many of the islets of Langerhans are independent of the acini and lie between the lobules in the connective tissue.

In category 4 the gland takes a much more mature form but the lobules are rounded, and relatively small, and connective tissue is prominent in filling the interlobular spaces.

Category 5 is almost the mature picture but the lobules are still rounded and the interlobular connective tissue can be easily seen.

Category 6 represents the fully mature gland. The lobules are densely packed, the interlobular margins forming an almost straight-line geometric pattern.

Other features of the immature pancreas, such as the relative increase in amount of islet tissue and the presence of small plugs in the acini, were not used in the assessment as the distribution of these structures within the individual gland appeared to be very variable.

Sections were stained by Masson's trichrome method and the category of the pancreas was decided without previous knowledge of the age of the child. Care was taken to ensure that the sections were cut directly across the pancreas, for in the growing pancreas the lobulation at the growing edges is looser than in the centre and a tangential section gives a false impression of immaturity.

RESULTS

The interrelation of the ages of the children and the arbitrary categories of the connective tissue is illustrated by a spot-graph in Table 1, and the numerical distribution in different age groups in Table 2.

No mature glands were found below the age of 2 months, and five mature glands were found in the 122 examined below the age of 1 year.

Table 1. *Table showing the relationship between the age of the child and the histological grading of the pancreas in 200 children. Each spot represents a single case*

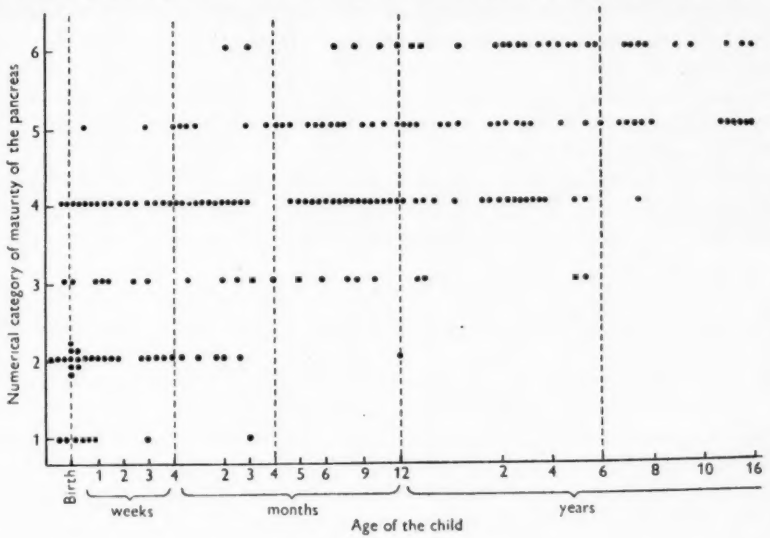

Table 2. *Table showing the distribution of cases in different age groups*

	Category of maturity (as in Pls. 1 and 2)						
	1	2	3	4	5	6	Total
Aged under 1 month	7	22	7	15	2	0	53
Aged 1–4 months	1	6	4	13	6	2	32
Aged 4–12 months	0	0	6	16	12	3	37
Aged 1–6 years	0	1	4	18	17	16	56
Aged 6–16 years	0	0	0	1	12	9	22

Category 4 was commonest in all age groups above the age of 1 month and below 6 years.

Of the fifty-three children beneath the age of 1 month, twenty-nine, i.e., more than half, were of category 1 or 2. The widest range was seen in the thirty-two cases between 1 and 4 months where every category of pancreas was seen. Over the age of 4 months types of category 5 first became common, twelve of the thirty-seven in the age group (4–12 months) being of that grade. In the age group 1–6 years the majority (fifty-one of fifty-six cases) were equally distributed between categories 4, 5 and 6, but a few more primitive glands were still present. The age group 6–16 years contained only twenty-two cases, but only one pancreas was below category 5.

DISCUSSION

The change in the general structure of the pancreas with age shows such variation as would be expected in any physiological process. It is also probable that the upper and lower limits of the normal distribution closely approach the abnormal. This

may be illustrated by a child dying at the age of 3 months with a pancreas of maturity 1, who was later found to be a mongol; no adequate cause of death had been found in a child dying at 1 year with a pancreas of maturity 2. Specimens between the ages of 1 month and 6 years fell mainly into category 4, but beneath the age of 1 year the numbers show that a greater amount of connective tissue may be expected.

The usefulness of the data obtained in this study lies chiefly in two directions:

(*a*) The provision of the figures for those who are only rarely called upon to report on the pancreas of small children.

(*b*) The establishment that a large amount of connective tissue is normal in many young infants, producing a histological picture which has been confused with the lesions seen in such diseases as congenital syphilis.

SUMMARY

The pancreas from 200 children, who died from diseases having no known lesion in the pancreas, was examined.

The glands were graded into six illustrated categories of maturity, based on the type of lobulation and the amount of connective tissue present.

The relationship of the graded maturity with age has been analysed and a spot-graph made showing the variation found at different ages.

It was shown that beneath the age of 1 month over half of the specimens had very loose lobulation and contained a very large amount of connective tissue, and glands showing an appearance differing considerably from the adult gland occurred through the whole of childhood.

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EXPLANATION OF PLATES

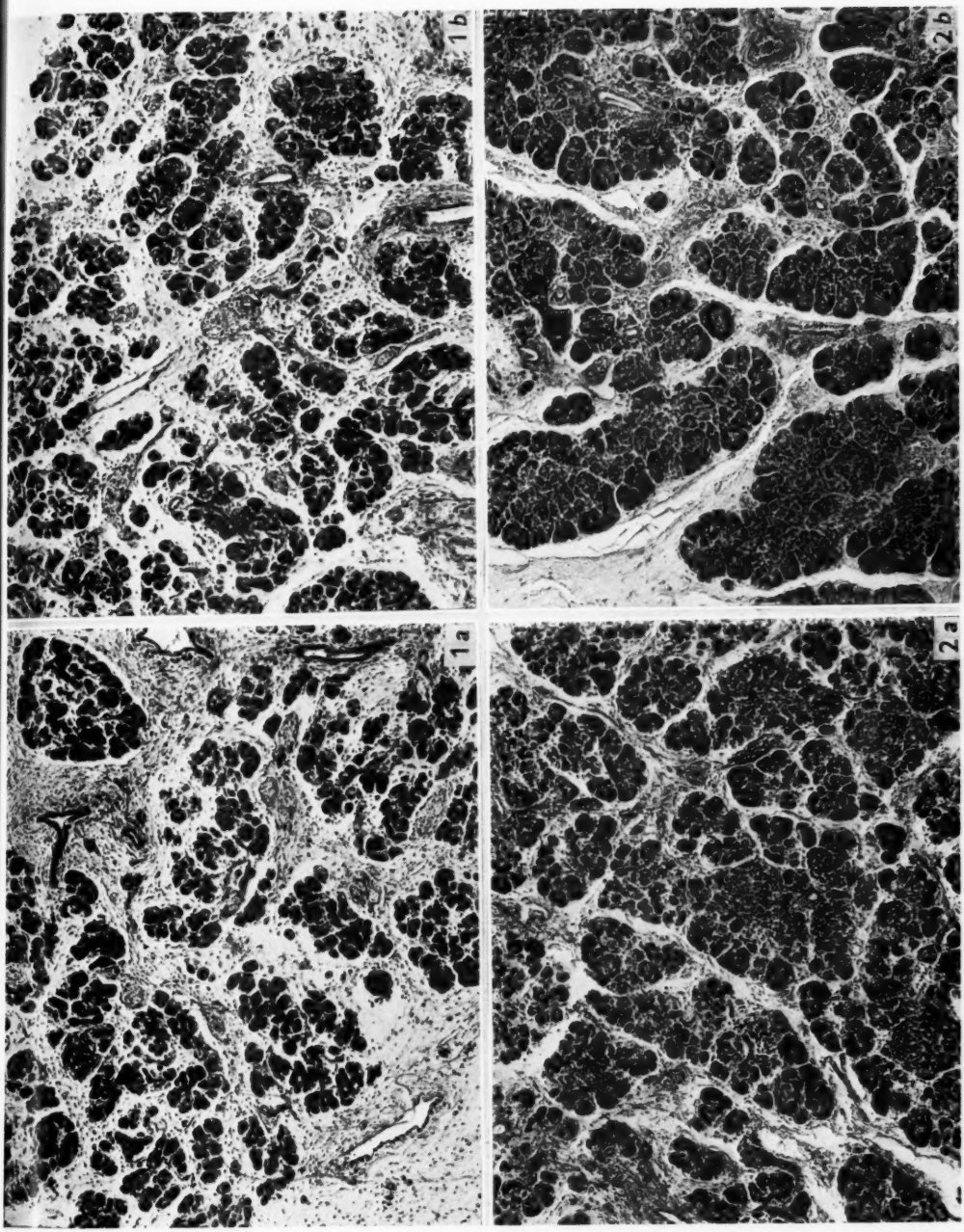
(The photomicrographs have been done by Mr F. Faulkner Taylor.)

Plate 1

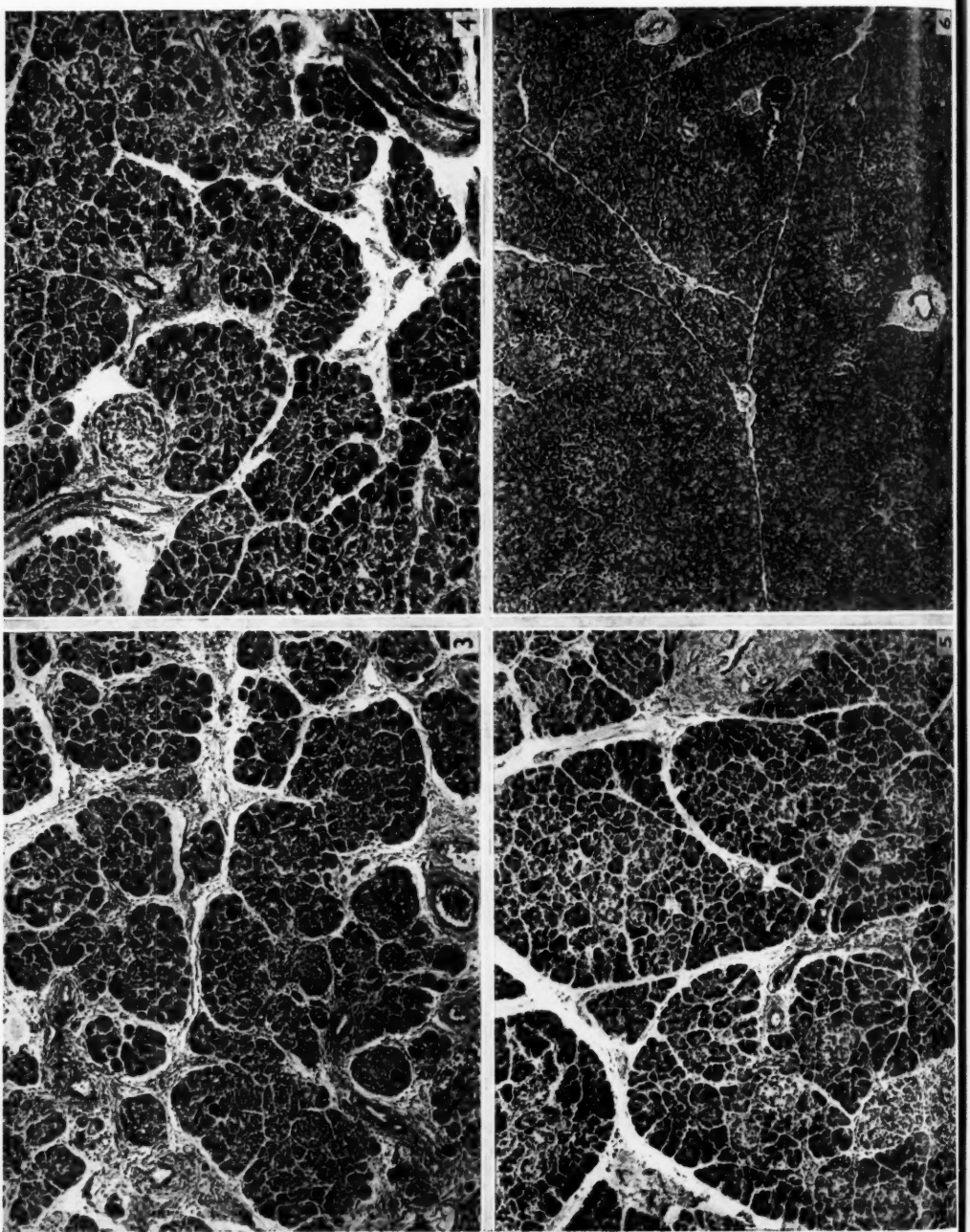
Illustrations of the pancreas showing the typical appearance in categories 1 (1*a* and *b*) and 2 (2*a* and *b*). Haematoxylin and eosin (× 74).

Plate 2

Illustrations of the pancreas showing the typical appearance in categories 3 (3), 4 (4), 5 (5), and 6 (6). Haematoxylin and eosin (× 74).



EMERY—POSTNATAL CHANGES IN LOBULATION AND CONNECTIVE TISSUE OF THE PANCREAS



EMERY—POSTNATAL CHANGES IN LOBULATION AND CONNECTIVE TISSUE OF THE PANCREAS

COMPARATIVE ANATOMY OF THE SUPERFICIAL VESSELS OF THE MAMMALIAN KIDNEY DEMONSTRATED BY PLASTIC (VINYL ACETATE) INJECTIONS AND CORROSION

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In spite of the fact that considerable work has been done on the circulation in the kidney, little attention has been paid to its superficial vessels. Morison (1926), in his paper on the renal circulation, illustrated (figs. 3, 4 and 5) the superficial veins of the kidney of man, dog and cat, as revealed by the celluloid injection and corrosion method. He described highly developed stellate veins in the cat and poorly developed ones in man, but gave no description of the superficial veins in the dog. Trueta, Barclay, Daniel, Franklin & Prichard (1947) studied the intra-renal circulation by various techniques and gave a detailed description of both the arterial and venous systems. They described (p. 164) superficial veins forming large venous trunks underlying the capsule in the kidney of the cat and comparable, but less developed, systems in man and the dog.

It was our purpose to study in some detail the superficial vessels of the mammalian kidney as seen after plastic injections and corrosion.

MATERIAL AND METHODS

A comparative study was made of the kidneys of two human newborn infants, twenty-one dogs, eight cats, twenty-eight rats, seven guinea-pigs, two sheep, one cow and one hedgehog. The animals used were of various sex and weight. The kidneys of the dog and of the cat were prepared by the method used for the dog's heart (Kazzaz & Shanklin, 1950). The method was slightly modified in the case of the other animals used, in that heparinization was omitted. Vinyl acetate (Ward's Natural Science Establishment, Rochester, N.Y.), of different colours, was injected into the renal artery under a pressure of 250–500 mm. Hg and into the renal vein under a pressure of 200–320 mm. Hg. The specimens were corroded in 2·5 % pepsin and 4·0 % hydrochloric acid.

OBSERVATIONS AND DISCUSSION

The veins constitute by far the largest component of the superficial vessels of the kidney. Three distinct patterns were observed in the arrangement of the superficial veins in the animals studied, and are typified by that found in the rat, the dog and the cat. All the superficial vessels described below were seen, in the injected but non-corroded specimen, to lie superficially under the capsule of the kidney.

I. *The superficial vessels in the rat* (Pl. 1, fig. 1). The superficial veins in the kidney of the rat constitute the simplest pattern found. They start under the capsule as spur-like projections, often with a bifurcation at their distal end, and pass

perpendicularly inwards through the cortex to join the interlobular veins, which in turn empty into the arcuate veins. None of the superficial veins passes for any considerable distance parallel to the surface of the kidney before turning inwards. Thus there is only one system of veins whereby the superficial and the deep veins empty into the arcuate veins before joining the renal vein. No arteries lie at the same level as or superficial to the superficial veins.

The kidneys of the guinea-pig, cow, sheep and hedgehog have superficial veins of this pattern.

II. *The superficial vessels in the dog* (Pl. 1, fig. 2). Here the veins are arranged in a stellate manner over the whole surface of the kidney. They converge to a number of central points and join together to enter the substance of the kidney perpendicularly and join the interlobular veins. The veins draining the medial side of the kidney are arranged in a fan-shaped manner and unite at the hilum to empty directly into the renal vein. This arrangement, however, is extremely variable. It may involve the veins near the hilum, or those over most of the kidney surface. There is an extensive anastomosis between all the superficial veins.

In some cases we observed one or more arteries emerging from the cortex and dividing into three or four branches which ramified underneath the capsule. In a few specimens arteries emerged from one surface of the kidney, passed round the lateral border and re-entered the substance of the kidney on the opposite surface (Pl. 1, fig. 2). Sometimes corresponding veins accompanied these arteries. Arteries of this kind were observed in the sheep and the cow. In one human kidney we found a single artery of this kind, while on the other side of the same subject there was a similarly arranged vein.

The human kidney has a system of stellate veins, similar to those in the dog, which drain into the interlobular veins. The degree of anastomosis, however, is less extensive and the distance that each vein runs over the surface of the kidney before penetrating it is much shorter than in the case of the dog. Unlike the condition found in the dog, the veins draining the medial part of the human kidney do not empty directly into the renal vein.

III. *The superficial vessels of the cat's kidney* (Pl. 1, fig. 3). A still more highly developed system of superficial veins is present in the kidney of the cat. It consists of four to six veins on each surface, which start at the lateral border and pass medially. They are radially arranged and drain into the renal vein at the hilum. All along their course they are joined on each side by numerous superficial subcapsular tributaries. From their deep surface many small veins pass perpendicularly inwards to join the interlobular veins, which in turn open into the arcuate veins. There are rich anastomoses between all the superficial veins in the cat as in the dog. From the above we conclude that the cat's kidney has two systems of veins—those that empty directly into the renal vein and those that join the arcuate vein before emptying into the renal vein. As in the rat, all the renal arteries lie deep to the superficial veins.

THE EARLY PRENATAL DEVELOPMENT OF THE HUMAN KNEE JOINT

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Descriptions of the embryonic development of the knee joint are readily available as in the recent comprehensive paper by Gray & Gardner (1950), but no details seem to have been published concerning the question of exclusion of the fibula from the knee joint during development. The present paper is concerned largely with this problem, and is confined to observations on man.

MATERIAL

For the present study serial sections of human embryos at the following stages were examined: 13 (two specimens), 15, 16, 17, 18, 19, 24, 25 and 28 mm. The 17, 18, 19, 24 and 28 mm. specimens were sectioned specially for the purpose of investigating the lower limb, and were stained with haematoxylin and eosin; the other specimens form part of the collection in the Department of Anatomy, University of Sheffield.

Wax-plate reconstruction, as utilized by Fürst (1925) and Langer (1929) in studying the early knee joint, has not been employed because the boundaries selected as the limits of the developing condensations at early stages depend in no small measure on personal interpretation, the nature of the intervening tissues remains undisclosed, and the models illustrated in the papers of the above authors (who give no photomicrograph of any of their sections) give a very misleading appearance of marginal definition.

OBSERVATIONS

In two embryos of 13 mm. the blastema had not become differentiated regionally. In a 15 mm. specimen condensations were well defined and the tibia was directly in line with the femur. The fibula was lateral to the femoro-tibial axis and appeared to reach the same level as the upper surface of the tibia. As only every fifth section was available, however, this embryo is not illustrated or described further in this paper.

In a serially sectioned embryo of 16 mm. the legs had been cut obliquely. The fibula had now lost its eccentric position and was situated under the femur. The tissue intervening between the femur and fibula was arranged as a three-layered interzone, as described in many joints (Haines, 1947, after Bernays). It consisted (Pl. 1, fig. 1) of two thick chondrogenic layers and a thin, looser, middle layer; in some sections, however, the middle layer was ill-defined and the whole interzone here appeared homogeneous. The fibula was identified from its relationship to the main nerve trunks and from the fact that it was seen first on examining the slides, beginning at the caudal end of the embryo; the arrangement was also checked from serial projection drawings, and, in any case, a similar interzone was found between the femur and the other element (tibia) in more cephalic sections.

The left lower limb of an 18 mm. embryo was sectioned serially at 9μ approximately in a coronal plane. At this stage the femoral condyles overhung the tibia and fibula on each side. The upper end of the fibula reached, or even exceeded, the same level as that of the tibia but appeared bent backwards (Pl. 1, fig. 2).

In the left leg of a 17 mm. embryo sectioned at 9μ the tibia was found to have a well-marked condensation projecting between the femur and the upper end of the fibula (Pl. 1, fig. 3), thus dividing the femoro-fibular interzone into femoro-tibial and tibio-fibular portions. A similar appearance, although not so distinct, was seen in the left leg of a 19 mm. embryo sectioned at 10μ (Pl. 1, fig. 4).

In a 24 mm. specimen sectioned at 10μ the fibula appeared to have retained its earlier eccentric position in relation to the femur and was thus only partially separated from that element by tibial tissue (Pl. 1, fig. 5).

At 25 mm. a well-defined three-layered interzone was found between the femur and tibia. Only every tenth section was available, however, and on this account it was not possible to determine the relationships of the upper end of the fibula.

By 28 mm. a three-layered superior tibio-fibular interzone was present; cavitation does not occur at this site until about 60 mm. (Gray & Gardner, 1950).

DISCUSSION

The present series does not elucidate the method of initial condensation of the tibia and fibula. Bardeen (1905) concluded that 'it is not clear whether or not the anlage of the tibia and fibula also begins as a continuous tissue sheet [as the foot does] which becomes divided, by ingrowth of blood-vessels, into tibial and fibular portions'. According to McDermott (1943), such is the case, for 'the tibia and the fibula are now [47–51 days] recognized as having become separate from one another after having arisen as a single sheet of condensed blastema [38–39 days]', whereas the anlagen of the femur and tibia 'have always been separate'.

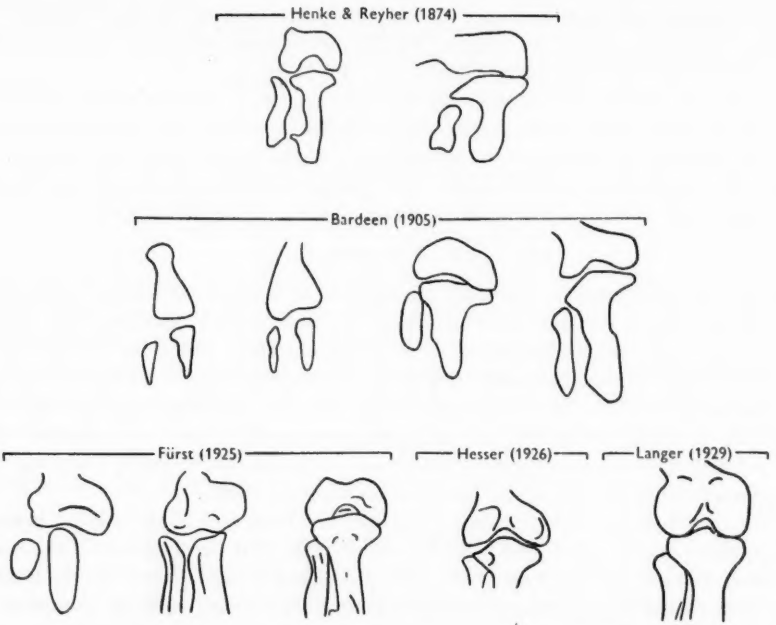
Bardeen (Text-fig. 1) found that at 11 mm. the tibia and fibula pointed towards the medial and lateral femoral condyles respectively 'but the long axis of the femur much more nearly meets that of the tibia than that of the fibula'. As the head of the tibia enlarged (14–20 mm.) its cephalic end was carried towards the centre of the distal end of the femur 'while the head of the fibula is pushed toward the side' so that 'the long axis of the fibula comes to point lateral to the extremity of the femur'. These appearances were also shown in Fürst's (1925) embryos, but in the present series, although the tibia was in line with the femur, the position of the fibula was less predictable, being lateral to the femoro-tibial axis at 15 and at 24 mm. stages.

Henke & Reyher (1874) appear to have been the first writers to describe and illustrate a femoro-fibular connexion in the normal human embryo (Text-fig. 1). In describing a 'fifth week' embryo, apparently 18 mm. in length, they state that the lateral femoral condyle does not quite touch the lateral tibial condyle (which seems to have been well formed), but is in connexion with the fibula, which is relatively long in the upward direction. The nature of the intervening tissues is obscure because these authors provided only line sketches.

Bardeen's (1905) reconstructions seem to confirm a close association between the femur and fibula at an early age, e.g. 14 mm. (Text-fig. 1). Bardeen noted that

during a period of rapid development (15–20 mm.) the femur, tibia and fibula may 'become temporarily distorted by resistance at the ends' and 'this is often especially marked in hardened specimens'. This appearance (whether natural or an artefact) was noticed in the present series and has been seen by a number of workers.

Fürst (1925), subsequent to similar work on the ox and to his thesis on the development of the popliteus, illustrated wax reconstructions of the human knee joint at early stages, but his work seems to have been overlooked by subsequent writers. At 14 mm. the fibula was separated from the lateral femoral condyle by a distinct



Text-fig. 1. Outlines of early knees, from drawings and reconstructions by various authors. To facilitate comparison the diagrams have been arranged so that each appears to be of the right knee as seen from the front. The two illustrations from Henke and Reyher are from the same lower limb, the second being described as a section nearer the median plane. In Bardeen's series there is a progressive lateral movement of the leg elements in relation to the lower end of the femur. The close relationship between the developing fibula and femur is shown clearly in the first figure from Henke and Reyher, in Bardeen's 14 mm. stage, and in Fürst's 20 mm. stage.

interval but, at 20 mm., the upper end of the fibula lay just under that condyle and was bent laterally and upwards as a 'processus capituli fibulae' (Text-fig. 1). At 25 mm. the fibula was separated from the femur by the lateral tibial condyle, but the tibia and fibula are described as not being yet in contact with each other. The photographs in Fürst's paper are of reconstructions only.

McDermott (1943) describes a femoro-fibular articulation in 'the brief period of time during which the fibula and the tibia are one blastemal mass, before there is any evidence of a joint space', but he does not illustrate this stage.

Other authors have not been able to confirm the existence of a femoro-fibular connexion: this is not surprising owing to the transitory nature of this stage. Langer (1929) describes and illustrates a model of a 24 mm. embryo in which the fibula is separated from the femur by the lateral tibial condyle (Text-fig. 1); and he adds that 'also in younger embryos, which I examined for this appearance [of a femoro-fibular articulation] I cannot ascertain that the upper end of this bone-anlage [fibula] reaches the level of the tibial condyles'. In the 18 mm. specimen of the present series the fibula reached, or even exceeded, the level of the tibia.

Gray & Gardner (1950), in their examination of the prenatal knee joint, did not observe 'the fibula enter into the formation of the knee joint'. Similarly, Moll,* in the course of investigations on the development of the knee joint, has not seen such a stage.

Stages found in the present series, however, confirm the existence of femoro-fibular tissue devoid of tibial material in the young embryo, and it has been pointed out in the present paper that a three-layered femoro-fibular interzone† has been found. In general, the blastema remaining between two chondrific centres forms a disc or interzone which is primarily chondrogenic and which later becomes continuous with synovial mesenchyme. The interzone has been regarded as the first morphological indication of a joint (Haines, 1947), but it is not essential to joint formation since a femoro-patellar articulation develops without an interzonal stage (Gardner, 1950). Furthermore, certain interzones do not proceed to joint differentiation but rapidly lead to fusion, e.g. that between the scaphoid and centrale in the human carpus, and the interzones associated with accessory carpal elements. In interzones at sites where postnatal articulations are not found in the human, processes other than fusion may occur, e.g., the interzone between the triquetrum and styloid process of the ulna becomes incorporated in the medial ligament of the wrist (Whillis, 1940). In the knee, the femoro-fibular interzone becomes invaded by tibial tissue; evidence is not available as to whether the lateral tibial condylar extension develops as an outgrowth from the tibia, or whether it condenses independently and then unites with the tibia. The appearance resembles that seen by the writer in an ulnar styloid process at 17 mm. which seemed likely to have been a separate condensation. Furthermore, a secondary union is not uncommon, occurring habitually between the scaphoid and centrale, and is found in the third metacarpal styloid process in nearly 70 % of cases (Dwight, 1907); the latter has been seen separate at 24 mm. by the present writer.

Inadequate growth of the lateral tibial extension may be expected to alter the width of the knee joint fissure; in the majority of cases (58 % of 150 knee joints) the lateral part of the joint fissure is wider than the medial (Bauer, 1931). Extreme width at the lateral side of the knee joint is occasionally met with, and is illustrated

* Stated at discussion of the present writer's paper, International Anatomical Congress, Oxford, 1950. At the same meeting R. Walmsley, on the other hand, stated that he had seen a femoro-fibular stage. Grünbaum (1892), who cites Leboucq, Bernays, and Gegenbaur as favouring contact between the fibula and femur, denies that this occurs; he illustrates only two specimens, however, one of which is at a relatively late stage. In this matter positive evidence is more significant than negative findings.

† The term femoro-fibular 'articulation' may be used, since the name *articulation* (or *joint*) is applied 'to the connexion subsisting in the skeleton between any of its rigid component parts, whether bones or cartilages' (Bryce, 1915).

in a skiagram by Köhler (1935). In cases where the tibia as a whole fails to develop the femoro-fibular interzone may cavitate; a femoro-fibular joint has been found in the adult by Ollerenshaw (1925) and by Dankmeijer (1935) in cases of absence of the tibia.

SUMMARY

1. A three-layered femoro-fibular interzone (of Bernays) has been found to occur in the human embryo at an early stage of development (about 7 weeks catamenial age).

2. Interzones take part in the chondrification process but are not an indication of joint differentiation since (*a*) they are not essential for the occurrence of joint differentiation, and (*b*) at certain sites they do not lead to joint differentiation. Both these points are exemplified in the embryonic knee.

3. Within a few days of its appearance the femoro-fibular interzone becomes invaded by tibial tissue.

4. In cases where the tibia fails to develop the femoro-fibular interzone is liable to cavitation.

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EXPLANATION OF PLATE

Fig. 1 is a sagittal section; figs. 2–5 are approximately coronal.

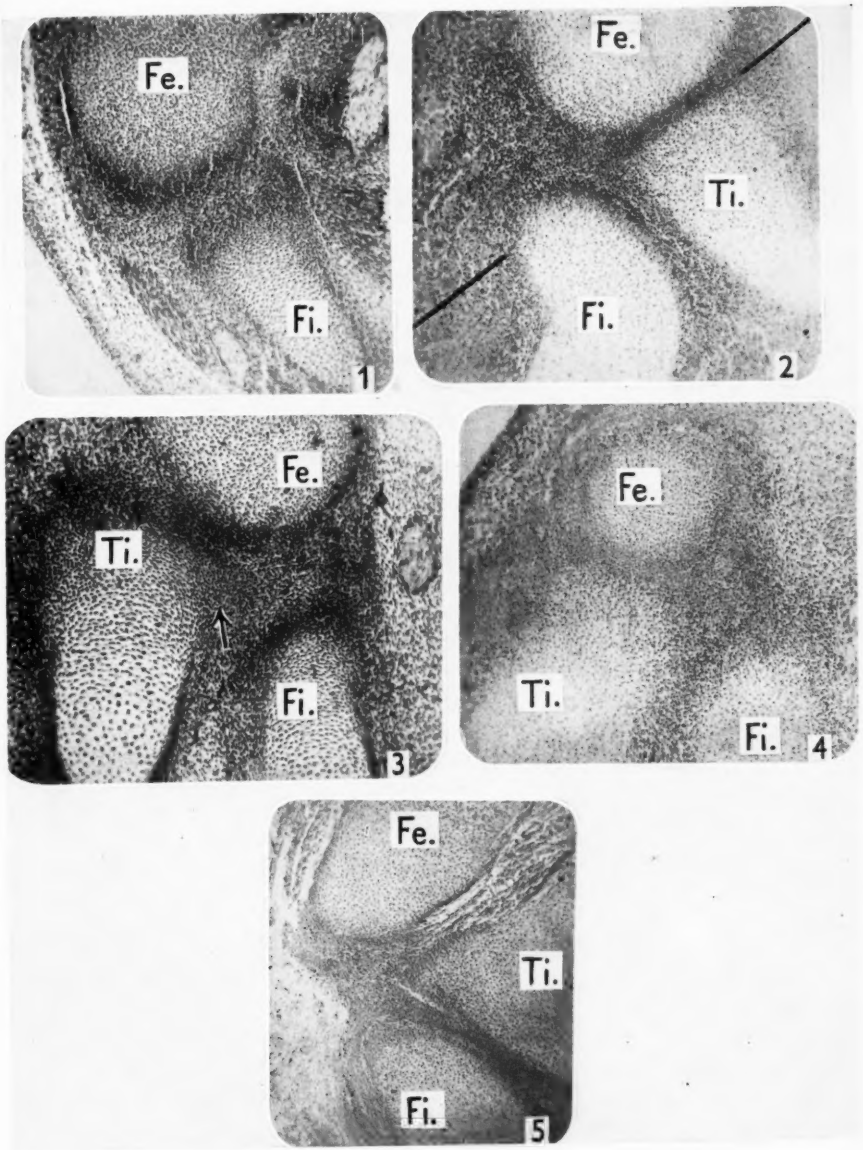
Fig. 1. 16 mm. Three-layered femoro-fibular interzone.

Fig. 2. 18 mm. The fibula reaches or even exceeds the level (indicated by the interrupted line) of the superior tibial surface.

Fig. 3. 17 mm. A lateral tibial extension (indicated by arrow) invades the femoro-fibular interzone.

Fig. 4. 19 mm. Showing an appearance similar to that of fig. 3.

Fig. 5. 24 mm. A later stage in the interposition of tibial tissue between femur and fibula.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THE BEHAVIOUR OF NUCLEAR STRUCTURES DURING DEPLETION AND RESTORATION OF NISSL MATERIAL IN MOTOR NEURONS

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INTRODUCTION

Electrical stimulation of motor cell axons is a convenient and effective method of producing extensive depletion of Nissl material in the parent cell bodies (Barr & Bertram, 1949). In the present study, this method has been used in order to observe the behaviour of certain nuclear structures during depletion and restoration of Nissl material. With the basic dye cresyl violet, components of the cell which contain large amounts of nucleic acid stain intensely, due to the phosphoric acid component of the nucleic acid molecule. The Nissl material of the cytoplasm is known to contain nucleic acid of the ribose type, in the form of nucleoprotein. The same type of nucleic acid is present in the large nucleolus, or plasmasome, so characteristic of the nerve cell nucleus. In the cat, the nucleolar satellite is the only intranuclear structure, other than the nucleolus, which appears well defined with the cresyl violet stain. The satellite, although much smaller than the nucleolus, is large enough to be readily examined in nerve cells of female cats, but lies at the limit of visibility, with an oil-immersion objective and ordinary illumination, in male cats (Barr & Bertram, 1949; Barr, Bertram & Lindsay, 1950). The nucleolar satellite, unlike the nucleolus and Nissl material, contains nucleic acid of the desoxyribose type. The neuron, therefore, lends itself especially well to a study of the relationship between cytoplasmic nucleoproteins and certain nuclear structures of similar chemical composition.

Interest in the present experiments arises from the fundamentally important role of the nucleic acids in cellular physiology. It is likely that information derived from the neuron concerning the structures responsible for the synthesis of cytoplasmic nucleic acids will apply to other cells characterized by large nucleoli and abundant cytoplasmic nucleoproteins. Obvious examples are to be found among rapidly growing and dividing cells such as occur in embryonic tissues, haematopoietic areas and malignant tumours.

MATERIALS AND GENERAL METHODS

In addition to three female cats, which served as unstimulated control animals, twenty cats were used in the experiments (seventeen females and three males). Under nembutal anaesthesia and with aseptic precautions, shielded bipolar platinum electrodes were applied to the right hypoglossal nerve as it crosses the carotid artery. The nerve was stimulated continuously for 8 hr. (50 cyc./sec.) by means of a vacuum tube stimulator with a square wave output, designed and constructed by

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Mr R. W. Luke, of the Department of Physiology. The initial output voltage was 10, with a gradual increase during the first hour to a maximum of 20 V. which was maintained during the succeeding 7 hr. There was a strong deviation of the tongue to the right throughout the period of stimulation.

Survival periods varied between 0 and 450 hr. (approx. 19 days). The animals were perfused, under nembutal anaesthesia, with isotonic 10 % formalin, according to the method of Koenig, Groat & Windle (1945). Serial, paraffin-embedded, 12μ sections were cut from the portion of the medulla containing the hypoglossal nuclei. The sections were stained routinely with cresyl violet (B.D.H.) in 0·5 % solution for 0·5 hr. at 56° C., followed by differentiation with alcohol under visual control. Sections of normal material, prepared by Mr Hugh Lindsay with the Feulgen and methyl green-pyronin methods, have been valuable in establishing the type of nucleic acid present in the nucleolar satellite.

SPECIFIC METHODS AND RESULTS

A. *Depletion and restoration of Nissl material*

A detailed description of changes in the cytoplasm, and the bearing of these observations on the important problem of the function of Nissl material, will be presented in a separate report. For our present purpose it is sufficient to note the following main points.

The alterations in the cells of the right hypoglossal nucleus (stimulated side) were assessed against the appearance of the cells in the left hypoglossal nucleus (unstimulated control side) in each animal. There was no visible change in the Nissl material in the two animals killed at the conclusion of stimulation. From 12 to 72 hr. following stimulation a progressive depletion of Nissl material occurred. The Nissl bodies became smaller until, in many cells, the Nissl material took the form of fine granules scattered diffusely and evenly throughout the cytoplasm. A cell with full complement of Nissl material is shown in Pl. 1, fig. 1. Cells with moderately severe depletion of Nissl material are illustrated in Pl. 1, figs. 2, 3.

When the change was at its maximum, the cells of the experimental side appeared a little larger than the cells of the control side. Possibly some dilution of the cytoplasm occurred, similar to that characteristic of axon reaction (Barr & Hamilton, 1948). However, the loss of stainable substance in the cytoplasm was too extensive to be accounted for by dilution alone. A considerable decrease in the total amount of Nissl material in the cell body must have occurred. This has been demonstrated quantitatively, under somewhat similar circumstances, by Hydén (1943) and Hamberger & Hydén (1945) using the method of selective absorption in the ultraviolet range.

A recovery phase followed, which lasted for approximately 14 days. Restoration of Nissl substance throughout the cytoplasm was preceded by the appearance of deeply basophil material at the nuclear membrane. These nuclear caps increased in frequency and size from the second day following stimulation well into the recovery period. Pl. 1, fig. 2, shows thin nuclear caps at several places along the nuclear membrane. The nuclear cap is better developed in Pl. 1, fig. 3. As recovery proceeded, the perinuclear region of the cytoplasm became filled with densely stained

particulate material (Pl. 1, fig. 4). Later, the entire cytoplasm became more deeply stained than in control cells, the normal Nissl pattern and density of staining being gradually restored. It is significant that restoration of the Nissl material proceeded from the nuclear membrane toward the cell membrane. All cells recovered.

The broken line in Text-fig. 3 represents the approximate time course of depletion and restoration of Nissl material, based on a careful examination of 500 nucleoli-containing cell sections in the control and experimental hypoglossal nuclei of each animal.

B. *Changes within the cell nuclei*

(i) *Nucleolus*

The nucleoli of normal neurons were Feulgen-negative and stained with the pyronin component of the methyl green-pyronin mixture. This was to be expected since it is well known that the nucleolus or plasmasome contains nucleic acid of the ribose type.

Table 1. *Changes in profile area of nucleolus (mean of fifty nucleoli ± S.E. of mean)*

Survival Time (hr.)	Sex	Profile area of nucleolus (μ^2)		Change (%)	S.E. of difference	Significant ? (critical value > 2·0)
		Control (left)	Exper. (right)			
Control	F.	13·0±0·4	13·4±0·4	+3·1	0·6	No
Control	F.	11·4±0·2	11·4+0·2	0·0	0·3	No
Control	F.	11·4±0·2	12·2±0·3	+7·0	0·4	No
0	F.	11·5±0·2	11·4±0·2	−0·8	0·3	No
0	F.	11·9±0·2	12·3±0·3	+3·3	0·4	No
12	F.	11·6±0·2	11·7±0·2	+0·9	0·3	No
24	F.	10·9±0·2	10·6±0·2	−2·7	0·3	No
36	F.	11·1±0·2	10·7±0·2	−3·6	0·2	No
48	F.	11·0±0·3	11·9±0·3	+8·2	0·4	Yes
60	M.	11·4±0·2	12·9±0·2	+13·2	0·3	Yes
72	F.	11·1±0·2	14·0±0·2	+26·1	0·3	Yes
72	M.	11·6±0·2	13·6±0·2	+17·2	0·3	Yes
84	F.	11·4±0·2	13·7±0·4	+20·2	0·4	Yes
96	F.	10·9±0·2	14·1±0·2	+29·3	0·3	Yes
108	F.	10·3±0·3	14·2±0·3	+37·9	0·5	Yes
120	F.	10·7±0·2	13·5±0·2	+26·2	0·3	Yes
136	F.	11·4±0·2	13·9±0·3	+21·9	0·3	Yes
156	F.	11·4±0·3	13·7±0·3	+20·2	0·4	Yes
192	M.	11·3±0·2	14·9±0·3	+31·9	0·4	Yes
214	F.	12·6±0·3	14·8±0·3	+17·5	0·4	Yes
288	F.	11·7±0·2	13·2±0·3	+12·8	0·4	Yes
384	F.	9·9±0·2	10·2±0·2	+3·0	0·3	No
450	F.	12·4±0·2	12·9±0·3	+3·9	0·3	No

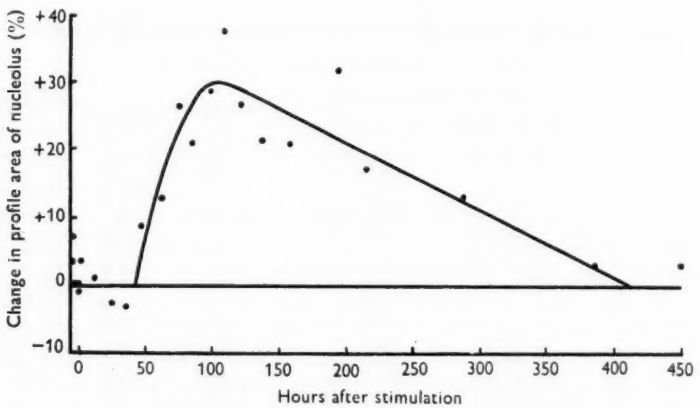
In sections stained with cresyl violet, the nucleoli of stimulated neurons were examined for alterations in size, position and internal structure.

Fifty nucleoli were measured in the control and experimental hypoglossal cell groups in each animal. Two diameters of each nucleolus were measured with a filar micrometer ocular, and the profile area determined by the formula for the area of an ellipse. The results are shown in Table 1. The variations in control animals and up to 36 hr. after stimulation were due to errors in sampling and measurement. However, in the interval between 48 and 108 hr. following stimulation, the nucleolus enlarged rapidly and significantly. There followed a gradual return to normal size which was reached about 400 hr. following stimulation. An approximation of the

time course of changes in nucleolar size may be obtained by constructing a smooth curve through the points derived from the data in column 5 of Table 1 (see Text-fig. 1).

Considering the series as a whole, the mean value for normal nucleolar profile area in the three male cats was $11{\cdot}4\mu^2$ and in the twenty female cats $11{\cdot}4\mu^2$. Dr W. H. Cook, working in this laboratory, had occasion to measure nucleoli in cells of the lateral geniculate body of the cat. The mean profile areas were as follows: females (seven animals), $4{\cdot}1\mu^2$; males (five animals), $4{\cdot}0\mu^2$. There appears, therefore, to be no significant difference in nucleolar size between the sexes.

Although the position of the nucleolus was variable in normal cells of the hypoglossal nucleus, it was usually near the centre of the nucleus, as seen in cell sections. No alteration in the position of the nucleolus in experimentally altered cells could be detected at any stage by general inspection. In order to make this observation



Text-fig. 1. Time course of nucleolar enlargement following stimulation of the axons of motor neurons.

more reliable, the distance between the centre of the nucleolus and the centre of the nucleus was measured, on camera lucida tracings, in a sample of fifty cells of the left and right sides, in three animals. The mean values were as follows: 48 hr.—control $2{\cdot}0\mu$, exper. $1{\cdot}8\mu$; 108 hr.—control $1{\cdot}8\mu$, exper. $1{\cdot}8\mu$; 288 hr.—control $1{\cdot}3\mu$, exper. $1{\cdot}4\mu$. These measurements confirm the general impression of a normal nucleolar position following stimulation of the nerve.

When stained with cresyl violet, nucleoli of normal cells in the control hypoglossal cell group showed little detail other than an occasional vacuole. The high nucleic acid content of the nucleolus caused it to stain so intensely with basic dyes that details of internal architecture were largely obliterated. The enlarged nucleoli of stimulated cells, however, showed extensive vacuolation. Either pre-existing vacuoles were unmasked by chemical changes in the swollen nucleoli or an increase in size and number of the vacuoles occurred. The latter alternative seems the more probable.

(ii) *Nucleolar satellite*

Since the nucleolar satellite was too small in nerve cells of male cats for satisfactory observation with the ordinary light microscope, only three males were included in the series. The results will be presented separately according to sex.

(*a*) *Females.* In cells of the control side, the satellite was roughly spherical in shape and approximately 1 μ in diameter (compared with a mean diameter of 4·0 μ

Table 2. *Incidence and position of nucleolar satellite in 12 μ sections containing the nucleolus and stained with cresyl violet (female cats)*

Survival time (hr.)	Hypoglossal nucleus	Position of satellite (%)			Total (%)
		Adjacent to nucleolus	Free in nucleoplasm	Adjacent to nuclear membrane	
Control	Left	64	9	2	75
	Right	62	11	1	74
Control	Left	37	11	9	57
	Right	39	9	11	59
Control	Left	51	12	6	69
	Right	54	9	6	69
0	Control	59	15	9	83
	Exper.	49	17	11	77
0	Control	43	15	7	65
	Exper.	40	18	6	64
12	Control	70	10	4	84
	Exper.	70	9	5	84
24	Control	61	9	7	77
	Exper.	55	18	8	81
36	Control	62	7	2	71
	Exper.	52	23	4	79
48	Control	72	10	1	83
	Exper.	54	29	2	85
72	Control	54	10	4	68
	Exper.	36	25	8	69
84	Control	42	17	4	63
	Exper.	24	41	7	72
96	Control	56	8	3	67
	Exper.	36	34	4	74
108	Control	55	7	2	64
	Exper.	40	28	4	72
120	Control	56	16	6	78
	Exper.	37	35	5	77
136	Control	68	11	7	86
	Exper.	38	42	7	87
156	Control	62	6	2	70
	Exper.	50	23	2	75
214	Control	63	7	10	80
	Exper.	41	27	8	76
288	Control	50	11	2	63
	Exper.	36	22	3	61
384	Control	53	10	5	68
	Exper.	50	12	8	70
450	Control	66	8	5	79
	Exper.	65	9	6	80

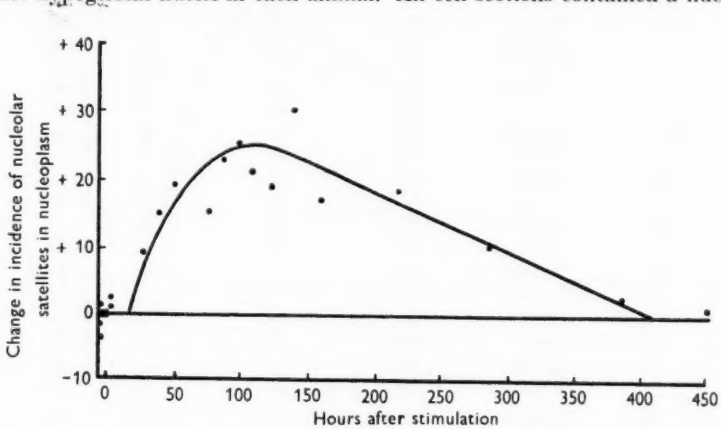
for the nucleolus). The satellite stained deeply with cresyl violet, due probably to its nucleic acid content. The satellite was Feulgen-positive and stained with the methyl green component of the methyl green-pyronin mixture. Its nucleic acid, therefore, is principally of the desoxyribose type, unlike the nucleolus and Nissl material, which contain ribose nucleic acid.

As shown in Table 2, the satellite of normal cells was situated most frequently adjacent to the nucleolus (Pl. 1, fig. 1). It was almost always spherical in this situation. When viewed tangentially with respect to the nucleolus, an exceedingly narrow clear interval was seen frequently between the nucleolus and its satellite. There may be a shallow concavity of the nucleolar surface at this point.

In a proportion of normal cells the satellite was free in the nucleoplasm, touching neither the nucleolus nor the nuclear membrane. Occasionally, such satellites departed from their usual spherical shape.

Still less frequently, the satellite lay adjacent to the nuclear membrane. In some instances the satellite was clearly flattened against the membrane.

The data in Table 2 are based on an examination of 500 cell sections in the right and left hypoglossal nuclei in each animal. All cell sections contained a nucleolus.



Text-fig. 2. Time course of movement of the nucleolar satellite away from the nucleolus following stimulation of the axons of motor neurons.

The frequency with which the satellite could be seen in 12μ sections stained with cresyl violet varied between 57 and 86 % (mean value 73 %). Slight differences in staining may have been responsible for this variation from one animal to another. There was likely one satellite to a cell, the organelle being invisible at times when eclipsed by the nucleolus, or when it was some distance from the nucleolus and in an adjacent section. Table 2 also shows the percentage incidence of the satellite adjacent to the nucleolus, free in the nucleoplasm, and adjacent to the nuclear membrane. While there was some variation from animal to animal, a definite pattern existed, typical of motor neurons of the hypoglossal nucleus. The mean values were: adjacent to nucleolus, 58 %; in nucleoplasm, 10 %; adjacent to the nuclear membrane, 5 %. (Total, 73 %.)

In the experimentally altered cells the most obvious change was the position of the satellite. During the second, third and fourth days following stimulation, an increasing number of satellites moved from the nucleolus for variable distances into the nucleoplasm (Table 2). The satellite is successively farther from the nucleolus in Pl. 1, figs. 2–4. The normal position was gradually restored during the succeeding 12 days.

Inspection of Table 2 shows that there was little change in the incidence of satellites adjacent to the nuclear membrane. The excursion of the satellite was for variable distances into the nucleoplasm, short of the nuclear membrane, and back to the nucleolus. The time course of satellite migration is best appreciated by constructing a smooth curve through points representing the difference in the incidence of satellites in the nucleoplasm in control and experimental cells (Text-fig. 2).

The satellite continued to stain deeply with cresyl violet throughout the period of experimental alteration. Although accurate measurement of such a small object proved difficult, the satellite clearly increased in size coincidentally with the increase in size of the nucleolus. Rarely, two enlarged satellites were encountered in experimentally altered cells.

It seemed of interest to learn whether the position of the satellite was influenced by the polarity of the neuron. It was found, from camera lucida tracings, that the axon arose predominantly from the medial and ventral aspects of the cell body. The nucleolar satellite, on the other hand, appeared to lie at random on all sides of the nucleolus. This random distribution applied to satellites adjacent to the nucleolus, free in the nucleoplasm and next to the nuclear membrane, in both control and experimentally altered cells. The polarity of the neuron, therefore, bore no obvious relationship to the position of the nucleolar satellite.

(*b*) *Males.* The nucleolar satellite in normal hypoglossal motor neurons of male cats was so small that there was difficulty in identifying it with certainty except in a small proportion of cells. This statement applies to the examination of cells with the ordinary binocular light microscope, with a Leitz fluorite oil-immersion objective 95×, aper. 1·32, and 10× oculars.

Table 3. *Incidence and position of nucleolar satellite in 12 μ sections containing the nucleolus and stained with cresyl violet (male cats)*

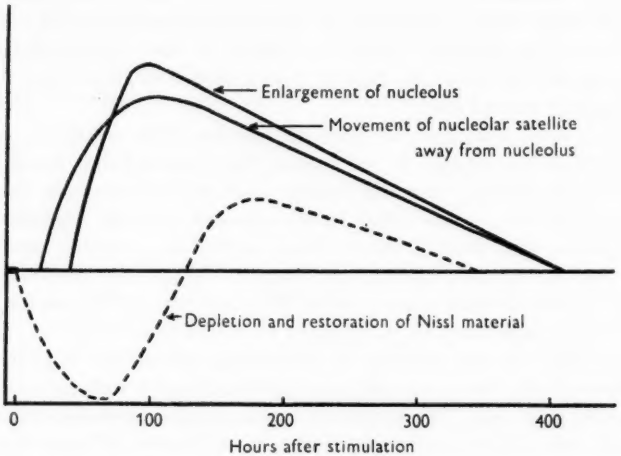
Survival time (hr.)	Hypoglossal nucleus	Position of satellite (%)			
		Adjacent to nucleolus	Free in nucleoplasm	Adjacent to nuclear membrane	Total (%)
60	Control	5	2	0	7
	Exper.	16	8	0	24
72	Control	2	1	0	3
	Exper.	12	7	0	19
192	Control	4	1	0	5
	Exper.	24	14	0	38

The figures in Table 3, based on an examination of 500 cells in each instance, are only approximate because of the minute size of the satellite in the male. However, comparison of the data for normal cells in Tables 2 and 3 clearly illustrates the difference in nuclear morphology according to sex. Pl. 1, fig. 5, represents a typical normal neuron in the male cat.

Following stimulation, the satellite of male cells enlarged so that the incidence of visible satellites increased. This is illustrated by the data in Table 3 (still approximate) for the incidence of satellites in male cells following stimulation. Occasionally, the satellite in the stimulated male neuron approached the size characteristic of the female. A cell in a male cat, with an enlarged satellite following stimulation, is shown in Pl. 1, fig. 6.

Finally, during the later stages of recovery of the Nissl material in both sexes, the basophilia of the nucleoplasm increased. This may be due to an increase in the nucleic acid content of the nucleoplasm.

The time course of changes in the content of Nissl material in the cytoplasm, enlargement of the nucleolus and changes in the position of the nucleolar satellite may be compared in Text-fig. 3.



Text-fig. 3. Combined curves of depletion and restoration of Nissl substance, nucleolar enlargement and movement of nucleolar satellite away from the nucleolus, following electrical stimulation of axons of motor neurons.

DISCUSSION

The morphological changes within the nucleus during depletion and restoration of Nissl material are consistent with the view that the nucleolus and associated structures play an important role in the synthesis of cytoplasmic nucleoproteins (Caspersson, 1947).

Cytoplasmic nucleic acid of the ribose type is abundant in cells which elaborate or maintain large amounts of protein. Typical examples are embryonic cells (Brachet, 1947), cells in haematopoietic tissues (Thorell, 1944), malignant cells (Caspersson & Santesson, 1942; Biesele, 1944; Stowell, 1947), serous secreting gland cells (Caspersson, 1947) and the neuron (Hydén, 1943). In each instance the cell is characterized also by a large nucleolus containing abundant nucleic acid of the ribose type.

The changes in the nucleolus are explicable if it is assumed that depletion of the

Nissl material, and in particular the ribose nucleoproteins of the Nissl material, serves as a stimulus to the nucleolus to elaborate substances necessary for the replacement of the depleted nucleoproteins. According to Hydén (1943, 1947), the nucleolus and associated structures, under similar circumstances, are the site of an increased level of ribose nucleic acid synthesis and also participate in the formation of simple proteins of the histone type, containing hexone bases. These substances, especially the histones, are thought by Hydén to pass from the region of the nucleolus toward the nuclear membrane. Cytoplasmic ribose nucleic acid and nucleoproteins are then laid down in the cytoplasm adjacent to the nuclear membrane. The enlargement and vacuolation of the nucleolus seen in the present experiments may be interpreted as a morphological expression of its increased activity. The chemical reactions necessary to the replacement of the cytoplasmic material appear to gain such momentum that the nucleic acid and nucleoproteins in the cytoplasm attain a level higher than normal before the cytological picture typical of control cells is restored.

The term 'nucleolar satellite' is used provisionally until more information concerning this interesting sex-influenced structure becomes available. This subject is considered in some detail elsewhere (Barr *et al.* 1950).

The behaviour of the nucleolar satellite during altered states of cell metabolism is worth close scrutiny. The movement of the satellite in the nucleoplasm is slow. Two weeks or more may be required for the satellite to make its full excursion for variable distances into the nucleoplasm and back to the nucleolus. If all material in the nucleus were visible and changes in the viscosity gradient between nucleolus and nuclear membrane known, a simple physical explanation for the movement might be apparent. Available evidence points to the flow of histones and nucleic acid from the nucleolus to the nuclear membrane. This flow, when accelerated, might carry the minute satellite along toward the nuclear membrane.

The increase in size of the nucleolar satellite during the restoration of Nissl substance suggests that the satellite may play an active role in the synthesis of some cytoplasmic constituent. If this should prove to be the case there may be a quantitative difference in some aspect of cell metabolism according to sex.

There is an interesting parallel between the behaviour of the nucleolar satellite in the cat's nerve cell and that of certain heterochromatic granules in the toad's egg (Painter & Taylor, 1942). During the development of the ovum, several hundred nucleoli appear which line up along the inner surface of the nuclear membrane. Each nucleolus has attached to it a minute heterochromatic granule which is located between the nucleolus and nuclear membrane. This morphological appearance of the nucleus is characteristic of the stage when large amounts of ribose nucleic acids are being laid down in the cytoplasm of the maturing ovum. One cannot fail to see certain parallels between the intimate relation of the multiple heterochromatic granules and the nuclear membrane in the toad's egg, and the migration of the nucleolar satellite toward the nuclear membrane in the cat's neuron, during a period of intense synthesis of cytoplasmic nucleic acid in both instances.

Finally we wish to point out that the cause of the severe depletion of Nissl material, following electrical stimulation of axons, is still in doubt. Liu, Bailey & Windle (1950) feel that damage to the axons by the stimulating current may be

responsible. However, the time course of the cell changes is shorter than one would expect were the phenomenon simply one of axon reaction. Further, characteristic chromatolysis has been observed in the absence of Wallerian degeneration distal to the site of application of the electrodes. Hydén (1943) described similar changes in spinal cord cells of guinea-pigs following exercise to exhaustion. Chromatolysis of cells of the cochlear ganglion in guinea-pigs was found by Hamberger & Hydén (1945) following exposure of the animals to loud sounds. It is tempting to think, therefore, that intense activity of the cells of the hypoglossal complex may have been at least partially responsible for the depletion of Nissl material which was so conspicuous in these experiments. Regardless of the cause of the depletion of Nissl substance, axon stimulation is a very useful means of producing extensive alterations in the metabolism of the neuron. The application of cytochemical methods to cells so altered may throw new light on the chemical reactions involved in the degradation and synthesis of the complex Nissl substance.

SUMMARY

1. Depletion of the Nissl material of motor neurons was produced by electrical stimulation of the cat's hypoglossal nerve. Chromatolysis in stimulated cells was first seen 12 hr. following stimulation, and increased progressively to the 72nd hour. A recovery period followed, complete at approximately 384 hr. The cells passed through a hyperchromatic stage before the normal Nissl pattern was restored.

2. The nucleolus increased in size progressively from 48 to 108 hr. following stimulation. Normal size was regained by 384 hr. Vacuolation was more apparent in the enlarged nucleoli than in nucleoli of unstimulated cells. Nucleolar position was unaltered.

3. The nerve cells of female cats contained a nucleolar satellite, approximately 1 μ in diameter. On the basis of the Feulgen and methyl green-pyronin stain, the satellite contained desoxyribose nucleic acid in contrast to the ribose nucleic acid of nucleolus and Nissl material. The satellite was rarely visible in normal male nerve cells.

4. Following stimulation, the satellite tended to move from its usual position, adjacent to the nucleolus, towards the nuclear membrane. The normal pattern, as concerns position, was restored by the 400th hour. The satellite, in female and male cells, increased in size following stimulation of the neuron.

5. The observations are consistent with the view that the nucleolus and possibly its satellite play a prominent role in the synthesis of ribose nucleoproteins, which are an important constituent of the Nissl material of nerve cells.

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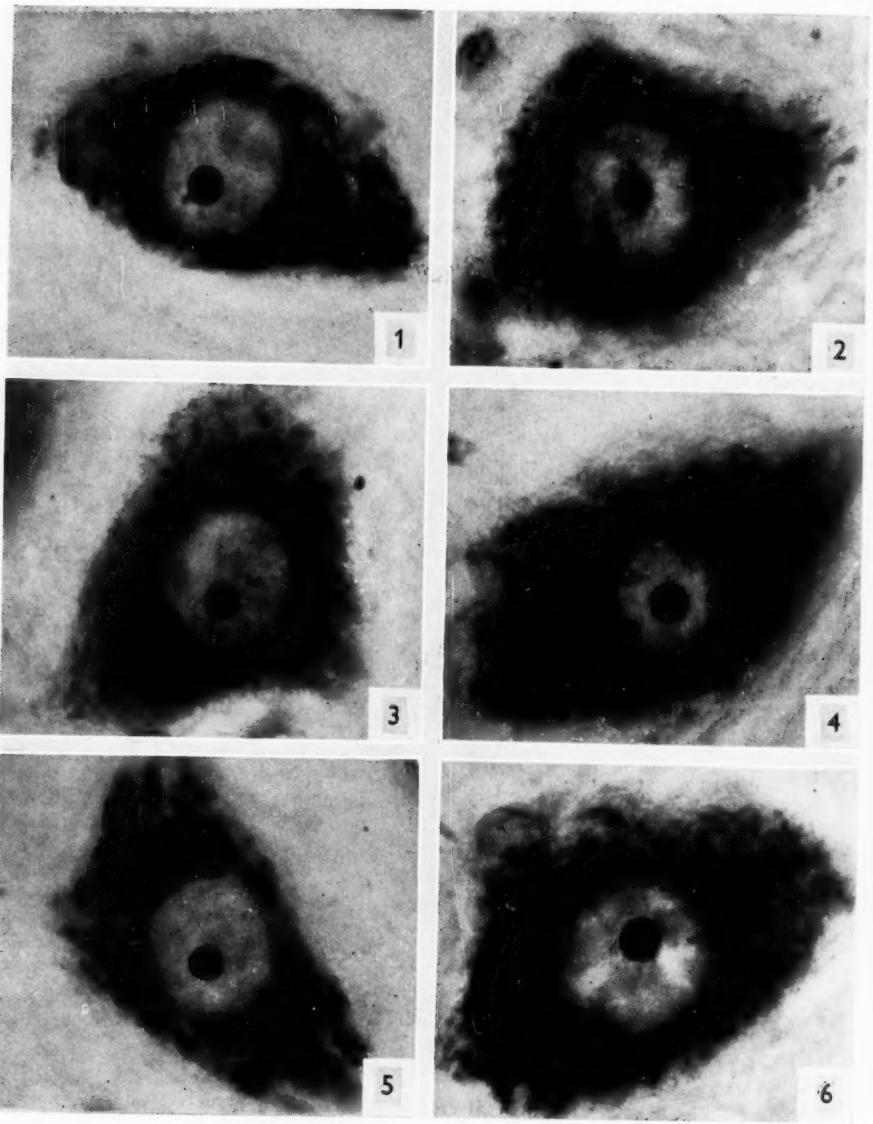

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EXPLANATION OF PLATE

Cresyl violet stain. Magnification × 1400.

Fig. 1. Normal hypoglossal motor nerve cell from control side of a female cat. The well-defined nucleolar satellite is seen adjacent to the nucleolus.

Fig. 2. Nerve cell of female cat 48 hr. following stimulation of its axon. There is moderately severe depletion of the Nissl material. Several thin nuclear caps lie adjacent to the nuclear membrane. The nucleolar satellite has begun to move away from the nucleolus.

Fig. 3. Nerve cell of female cat 72 hr. following stimulation of its axon. The degree of chromatolysis is similar to that shown in fig. 2, but the nuclear cap is more prominent and the satellite has moved farther into the nucleoplasm.

Fig. 4. Nerve cell of female cat well on the way to recovery 108 hr. following stimulation of its axon. The Nissl material is less dense at the periphery of the cytoplasm as compared with the perinuclear zone. The enlarged satellite is a considerable distance from the nucleolus.

Fig. 5. Typical normal male neuron from the hypoglossal nucleus. There is no visible nucleolar satellite.

Fig. 6. Neuron from a male cat 192 hr. following stimulation of its axon. A small nucleolar satellite is visible at about 3 o'clock not far from the nuclear membrane.

THE FIFTH INTERNATIONAL ANATOMICAL CONGRESS, 1950: ORGANIZATION AND LOGISTICS

By D. C. SINCLAIR

From the Department of Human Anatomy, University of Oxford

The fifth International Anatomical Congress was held in Oxford from 25 July to 28 July inclusive, and was attended by a record number of members. On the evening of their arrival, they were received in Christ Church by Viscount Addison, K.G., P.C., on behalf of His Majesty's Government. The next morning, when the Congress officially opened, the members were welcomed by the President, Prof. W. E. Le Gros Clark, F.R.S. In his opening address (*Brit. med. J.* 1950, **2**, 233) the President reviewed the progress made in the past decade in the many fields of activity in which anatomists are engaged, and emphasized the fundamental status in biology of the study of structure. At the University Convocation which followed, the honorary degree of D.C.L. was conferred upon Lord Addison, and of D.Sc. on Prof. G. W. Corner of Baltimore, Prof. M. W. Woerdeman of Amsterdam and Dr L. S. B. Leakey of Nairobi. In the afternoon the scientific sessions began, and lasted until midday on 28 July, with a break for recreation on the afternoon of the 26th. In the afternoon of the 28th the Congress dispersed after a brief closing address by the President. At this closing session a number of resolutions were adopted. It was decided to set up an international committee to consider the possibility of modifying the B.N.A. terminology to satisfy the diverse groups of workers who make use of it, and that an approach should be made to U.N.E.S.C.O. for funds to provide a paid secretary to this committe. Secondly, it was agreed to form another international committee to make a record of the sources of films which could be used in the teaching of normal anatomy, with a view to the formation of an international catalogue, and possibly an international lending library. The Congress expressed its sympathy with the relatives of Prof. H. C. Bazett, who had died on his way to attend the Congress. Finally it was resolved to congratulate Prof. F. Hochstetter and Prof. H. K. Corning on attaining their present venerable ages.

At the closing session, Prof. J. Benoit proposed that the next Congress should be held under the auspices of the Association des Anatomistes de langue Française in 1955 and suggested Algiers as the place of meeting; this proposal was carried by acclamation. Prof. N. L. Hoerr then stated that the American Association of Anatomists desired to issue a 'standing invitation' to anatomists to hold a subsequent Congress in the United States; this statement was also very warmly received. The probable date and place of the next Congress, and the body to be responsible for its organization, were thus fixed by a method that appears to be greatly preferred to the tedious process of forming international committees.

Such are the bare facts of the Congress; they have already been reported in greater detail (*Lancet*, 1950, **2**, 226; *Nature, Lond.*, 1950, **166**, 858). Many requests have, however, been received from overseas visitors that some effort should be made to uncover the machinery of the Congress, that some attempt should be made to peer,

as it were, into the maelstrom which eventually threw out on to a relative calm sea a Congress which actually floated. For these reasons it is felt that a breach with the traditional method of describing a medical Congress by its surface anatomy only may be pardoned, and that some interest may lie also in the description of the underlying topography.

The first International Anatomical Congress was held in Geneva in 1905, the second in Brussels in 1910, the third in Amsterdam (1930) and the fourth in Milan (1936). It was intended to hold the fifth in the United Kingdom in 1940, but owing to the war this proved impossible. The Anatomical Society of Great Britain and Ireland therefore decided to invite delegates from other nations to an International Congress to be held in Oxford under the Presidency of Prof. W. E. Le Gros Clark. A special Congress Committee was set up, consisting of the late Prof. A. B. Appleton, Prof. J. D. Boyd, Prof. J. C. Brash, Prof. F. Goldby, Prof. W. J. Hamilton, Prof. T. Nicol, Mr K. C. Richardson, Prof. J. M. Yoffey, Prof. J. Z. Young and Prof. S. Zuckerman. This committee advised on such subjects as finance, languages, special invitations, political considerations, etc., and appointed as Secretary and Treasurer to the Congress Dr A. G. M. Weddell. A preliminary estimate of the probable cost of the Congress was made, and an approach to the Royal Society resulted in the sum of £400 being made available to meet costs over and above those defrayed by the subscriptions, which were fixed at £2. 10*s*. per member and £1 per associate member. (It may be interpolated here that the whole of this £400 was in fact used.)

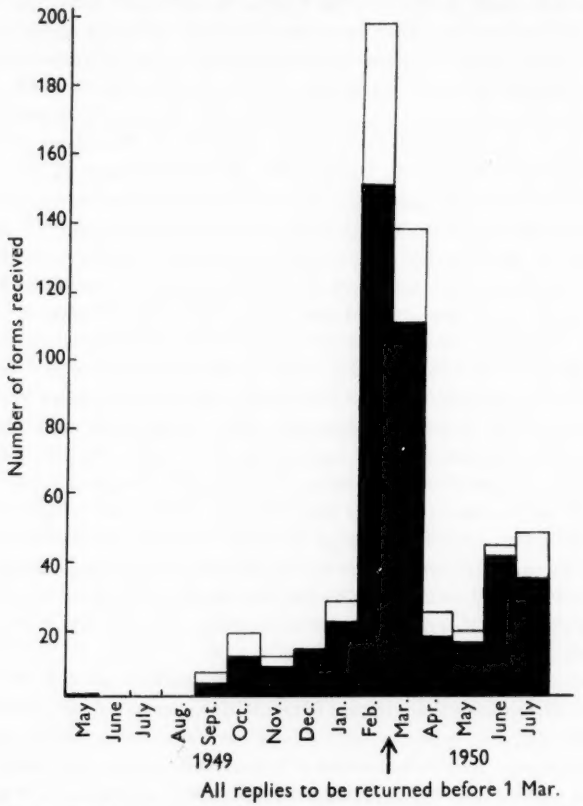
The detailed organization of the Congress came largely under the care of the Secretary and of the Congress Organizer, Miss Audrey Mayall. Advice of the greatest value was obtained from Captain E. W. Geidt, who was responsible for organizing the seventeenth International Physiological Congress at Oxford in 1947. The first difficulty arose over the invitations. There are only a few national Anatomical Societies, and it was important not to leave anyone out. Therefore, in addition to approaching these Societies, all Medical Schools and Biological Societies were circulated, as well as the senior scientific societies of every country. For this purpose, *The World of Learning* (2nd. ed., London, Europa Publ. 1948) proved to be indispensable.

The invitation forms (Appendix 1) were based on those used at the seventeenth International Physiological Congress; some of them were not satisfactory, and it is suggested that radical improvements might well be made. Thus, form F was of little use, and it was found that most of the members who had filled in this form did not avail themselves of the arrangements made for them, or that these were sometimes in conflict with their own private arrangements. Again, form E, dealing with the showing of cinematograph films, might well be combined with form C, dealing with communications. Most of the trouble, however, arose in connexion with forms C and D (communications and demonstrations), and an amended version of these two forms along the lines suggested in Appendix 1 is highly desirable.

The invitation forms were sent off in August 1949, with a request that all forms should be completed and returned not later than 1 March 1950. By this date only 278 replies (see Figs. 1 and 2) had been received. This occasioned no great concern, since data were available from the seventeenth International Physiological Congress to show that the physiological reaction to a dead-line of this nature was exactly

similar. However, it became necessary to send out reminder forms. In spite of this, a large number of completed invitation forms did not arrive until the end of June or later, thus complicating the arrangements for printing, etc., and necessitating the frequent revision of all working lists of members, communications, etc.

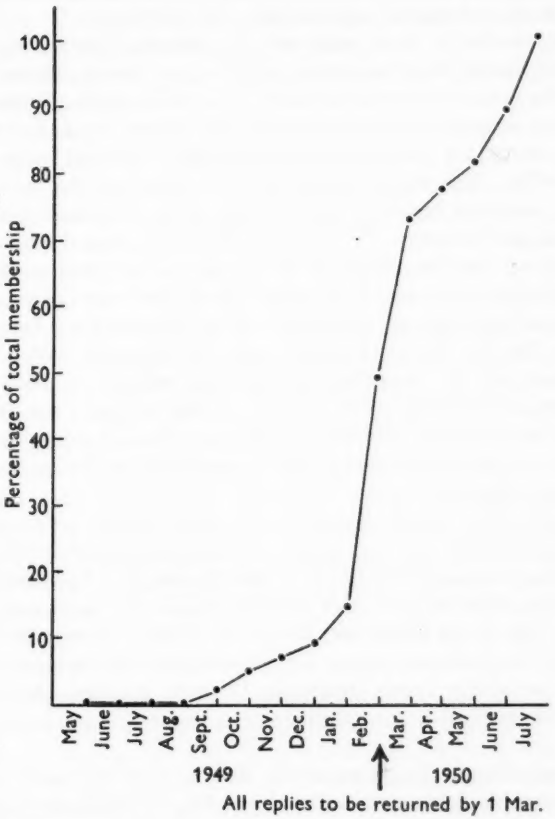
Problems relating to the question of printing were in fact greatly in evidence. In the parcel of documents handed to each member on registration were a name tag, a programme of scientific sessions, an entertainment and information guide, the



Text-fig. 1. Number of membership forms received in each month. The shaded areas represent members; the blank areas represent associates.

abstracts of the communications, a membership card, a local guidebook and map, and a copy of the History of the Teaching of Anatomy at Oxford, written by Drs H. M. Sinclair and A. H. T. Robb-Smith, and made ready specially for the occasion. The printing of many of these was conducted in a rush owing to the failure of members to be prompt in replying to the invitations. For this reason it was quite impossible to circulate beforehand the programme of scientific sessions, as it had been hoped to do; indeed, the copies of this programme only arrived from the printers two days before the Congress started. Nor was there enough money

available to print successive emended versions of any of the programmes, though a preliminary programme, containing as much information as possible, was in fact circulated to members some time before the Congress. In addition to all these items, the invitations, the acknowledgment forms, the reminder notices, the tickets for various functions, the text of the orations for the Convocation, and the signposts for demonstrations, buildings, lavatories, etc., had all to be printed. In connexion with



Text-fig. 2. Cumulative growth of membership expressed as percentage of total membership.

the signposting an estimate had to be made beforehand of how many signposts and notices constituted the minimum necessary to prevent people getting lost. Trial runs were in fact conducted, using a subject unfamiliar with the terrain and engaged in strenuous conversation throughout, in the manner of anatomists. The subject did not get lost, but several anatomists later did so.

A total of 569 people accepted the invitation to the Congress, but, as was inevitable, some found themselves unable to come, and the actual attendance was 535, representing 36 different countries (see Appendix 2). Even so, this figure was larger than

the attendance at any previous Congress, and the problem of accommodation was a considerable one. For the most part members were quartered in the Colleges of the University, hotel accommodation in Oxford being lamentably inadequate. So far as possible an attempt was made to billet members in Colleges reasonably near to the Science Departments, or in Colleges from which it was an easy matter to obtain transport to near the Science area. There were several last-minute cancellations of accommodation, and it is suggested that it might prove expedient on future occasions to impose a small booking fee to cover such a contingency.

It was early decided to have some sort of a Congress Centre, and thanks to the kindness of the Rhodes Trustees it was possible to use Rhodes House, the proximity of which to the Science departments made it an ideal choice. Hither the members were conducted on their arrival to register in the visitors' book, here were the bank, the stationery store, the post-office, the exhibition of medical books, the television set and the coffee. Here all the documents—all in an envelope with the name tag outside—were collected by every member, who could then book tickets for various entertainments and collect travel literature. Here were posted notices of additional scientific meetings and lists showing where each member was accommodated and which members had been unable to come. These lists were invaluable, and there was hardly a moment during the Congress when they were not being consulted by someone. In Rhodes House problems could be discussed with the appropriate Congress official, and the centre was kept in touch with all the buildings concerned in the Congress by telephone or by runner. A field telephone was installed for the occasion, and the statutory insurance of the wire where it crossed over a road was a heavy item; a loud-speaker system was arranged in the Centre itself and in each department concerned.

Approximately three months before the Congress opened, several subcommittees were formed to deal with separate aspects of the preliminary work: Communications, Demonstrations, Guides and Interpreters, and Hospitality. In addition, all arrangements for extra-scientific activities and entertainment came under the general supervision of the Local Organizer, Dr H. D. Darcus. It was found that in the business of all these subcommittees local knowledge was absolutely indispensable and scientific knowledge highly desirable. In short, it appears to be preferable to draw the chairmen of all such committees from the staff of the local Department of Anatomy.

The subcommittee on Communications had to deal with such matters as the organization of sessions to avoid, so far as possible, any clashing of interest, so that as few members as possible would have to be in two places at once. In all, there were 217 communications on the programme, and six lecture theatres were found necessary. The arrangement of sessions was somewhat hampered by the late arrival of forms and by cancellations. Papers were grouped under various elastic headings, and so far as possible papers with related interest were collected together in one session. The difficulty of classifying papers under any rigid system will be apparent from the list of communications (Appendix 3), which also demonstrates the wide range of subjects aired at the Congress. The largest single section was that of Neurology, with 65 papers, while General Anatomy had 47 papers, Embryology and Morphogenesis 34, Histology 33, and Physical Anthropology and Endocrinology

19 each. The large number in the section of Neurology necessitated the holding of two simultaneous Neurology sessions on three occasions, and the same thing had to be done twice with General Anatomy. This state of affairs suggested the possibility of holding symposia on given topics, and rejecting papers which did not fit in with the subject matter of the symposia. This was not in fact done, but it is clear that if in future Congresses the number of papers under a given heading rises much further it will be necessary to consider such a step. Abstracts of the communications were circulated to all members, but owing to the cost it proved impossible to publish any Proceedings of the Congress.

In each lecture theatre the potentialities of the lantern and its slide carriers had to be investigated, and practical testing of all lanterns, epidiascopes and projectors was found to be essential. Owing to the inadequacy of the form inviting members to give communications some difficulty was experienced over the various sizes of lantern slide to be projected; these were not always clearly specified. It is suggested that in view of the relative ease with which lantern slides can be made it would be desirable on future occasions to restrict the number of different sizes which can be shown. Projectionists had to be found for each theatre at each session, and arrangements had to be made for interpreters for the appropriate languages to be on hand at the crucial moments. The policy was to restrict interpretation to the question times allowed after each paper, and not to attempt to translate each paper into several languages, which would have been far beyond the local resources as well as too time-consuming. English abstracts of each paper were distributed at the beginning of each session where necessary. Only twenty minutes could be allotted to each communication and its discussion, and there was not enough time to translate the lengthy comments which were sometimes made. This of course raises the perennial difficulty common to all such occasions—that of inducing speakers to keep to the limits of their time and questioners to keep their remarks as succinct as possible. There appears to be no satisfactory solution which does not engender bad feeling, but the honorary chairmen of the sessions did their best—usually very successfully—to keep to the time-table. It was found an advantage to have on the chairman's table a copy of the rules for the chairman and a copy of the programme for each session, emended up to date. Messages could be relayed to all points by the loud-speaker system, and in each department was stationed a student helper deputed to write up messages on a blackboard sited just inside the door. This human element in an essentially electronic chain of communications occasionally proved its weakest link, since when anything specially interesting was going on in the lecture theatre the blackboard man might be found inside listening.

The Demonstrations subcommittee had its own problems. There were 90 demonstrations on the programme, and seven more eventuated on the first day of the Congress; these could not, of course, be put on the programme, and had it not been for a number of last-minute cancellations, might have proved impossible to accommodate. It was expected that most demonstrations would be set up on the day before the Congress opened, and on that day the microscopes were distributed and staff were available in each building, armed with sticky tape, cedarwood oil, drawing pins, etc., to assist members to set up their demonstrations. The timing of arrivals was, however, slightly awkward, and many members left themselves no

time to set out demonstrations that night. In fact, more than half the demonstrations did not make their appearance until the next day or later, after the scientific sessions had begun.

In view of the deficiencies in the form inviting members to give demonstrations, guesses had often to be made as to the amount of bench space required, but calculating from the figures given by some members, and allowing 2 ft. between microscopes in every case, the total requirements worked out at approximately 950 ft. (roughly 300 m.). This allowance appeared to be adequate, though individual demonstrators were sometimes cramped. The boundary between the space allotted to one demonstrator and the next was marked by a strip of white adhesive tape, which permitted local (and occasionally amicable) adjustments to be made between neighbouring demonstrators. The large amount of space required meant using four different buildings, and it proved impossible to avoid altogether that bugbear of all demonstrations, the long steep stairway of approach. Difficulties were also encountered in the grouping of demonstrations having related subject-matter. These difficulties were often forcibly resolved by such factors as the length of individual benches, the presence or absence of lighting and power plugs, etc.

Each demonstration received a number, and was labelled with the names of the members concerned. To facilitate signposting, the numbers ran consecutively in any given department, but since it was not possible to house all the demonstrations falling under one heading in the same department, this procedure meant that when the demonstrations were grouped together by subject-matter in the programme, the numbers were no longer consecutive, which tended to be confusing. In the circumstances there appeared to be no easy way out of this difficulty. A list of the demonstrations is given in Appendix 4. All demonstrations were on view continuously, and no special time could be spared to be set aside for viewing them. This necessitated the supervisory presence of a member of the Anatomy Department throughout the day in each of the rooms concerned; a system of reliefs was arranged for this duty.

The total number of microscopes requested was 384, or just over four per demonstrator. To meet this request, over 100 microscopes had to be borrowed from outside Oxford, and the transport of these arranged for; each microscope was insured during transit and while on exhibition at the Congress for a sum of £50. In the end, 402 microscopes from various sources were available, a reserve of 18 being kept for emergencies. The numbers of all borrowed microscopes and the numbers of their lenses were recorded, and their origin was denoted by different coloured labels to facilitate recognition and packing; the position of each microscope was charted on a plan. This last precaution was adopted to prevent unauthorized exchanges of lenses between neighbouring microscopes.

With regard to lenses, 81 oil immersions were asked for, and a total of 90 was obtained in order to keep a reserve. All requests for unusual lenses (e.g. 8 mm. objectives) were met locally with the exception of a number of 36 mm. objectives which were brought by the demonstrator concerned. All the 19 low-power binocular microscopes and dissecting lenses asked for were obtained locally, as were the two polarizing microscopes. One phase-contrast instrument was brought by

its proprietor, and another was available. Viewing boxes were extremely scarce, owing to the imminence of a Radiological Congress in London, but enough were obtained to satisfy the demand, which was for six.

The lighting of all microscopes was provided for by borrowing lights or by manufacturing them. The first alternative was unsatisfactory owing to the chaotic electrical systems prevailing locally, it proving inevitable that the plugs of lamps borrowed from one department would not fit the sockets of another, or that a different voltage would be involved. In the end, the majority of the extra lights required were made by mounting 40 W. frosted bulbs 2 ft. apart on wooden battens, and screening them by a series of simple folded metal plates with holes in the appropriate places. These lamps were connected in parallel and plugged in to power points; they proved quite satisfactory even for oil-immersion lenses.

Many of the difficulties on the demonstration side of the Congress would not have arisen had a limit been set upon the number of microscopes permitted to each demonstrator. It is difficult to persuade members that a few microscopes accompanied by a chart, with some photomicrographs, and additional slides available on request, make a bigger impression and are looked at by more people than a forbidding array of oil-immersion lenses all showing much the same thing. It is therefore suggested that in any future Congress it will be desirable to impose some numerical limitation.

The problems met with by the subcommittee on Guides and Interpreters were chiefly concerned with interpretation. The official languages of the Congress were English, French, German, Italian and Spanish, so that interpreters for these languages had to be found. It was of course preferable that they should have some medical or at least biological knowledge, so that the field was at once considerably narrowed, and the search for interpreters became an exhausting and eventually an expensive item. So much was this the case that in a future Congress it should probably be considered, before deciding on the official languages, what local facilities for interpretation exist. Eventually two interpreters in each language were found, while for French an additional three were used, the total of five being necessitated by the fact that from the programme it was possible that this number might be needed simultaneously during the scientific sessions. The most strenuous time the interpreters experienced was during the first arrival of members at the Congress centre. In addition to the interpreters, 15 medical students, who were paid a nominal fee for their services, acted as guides, runners, telephone orderlies, ushers, etc. All Congress officials and helpers were identified by different coloured arm-bands with their designation printed on them in black; the interpreters bore simply the name of their language.

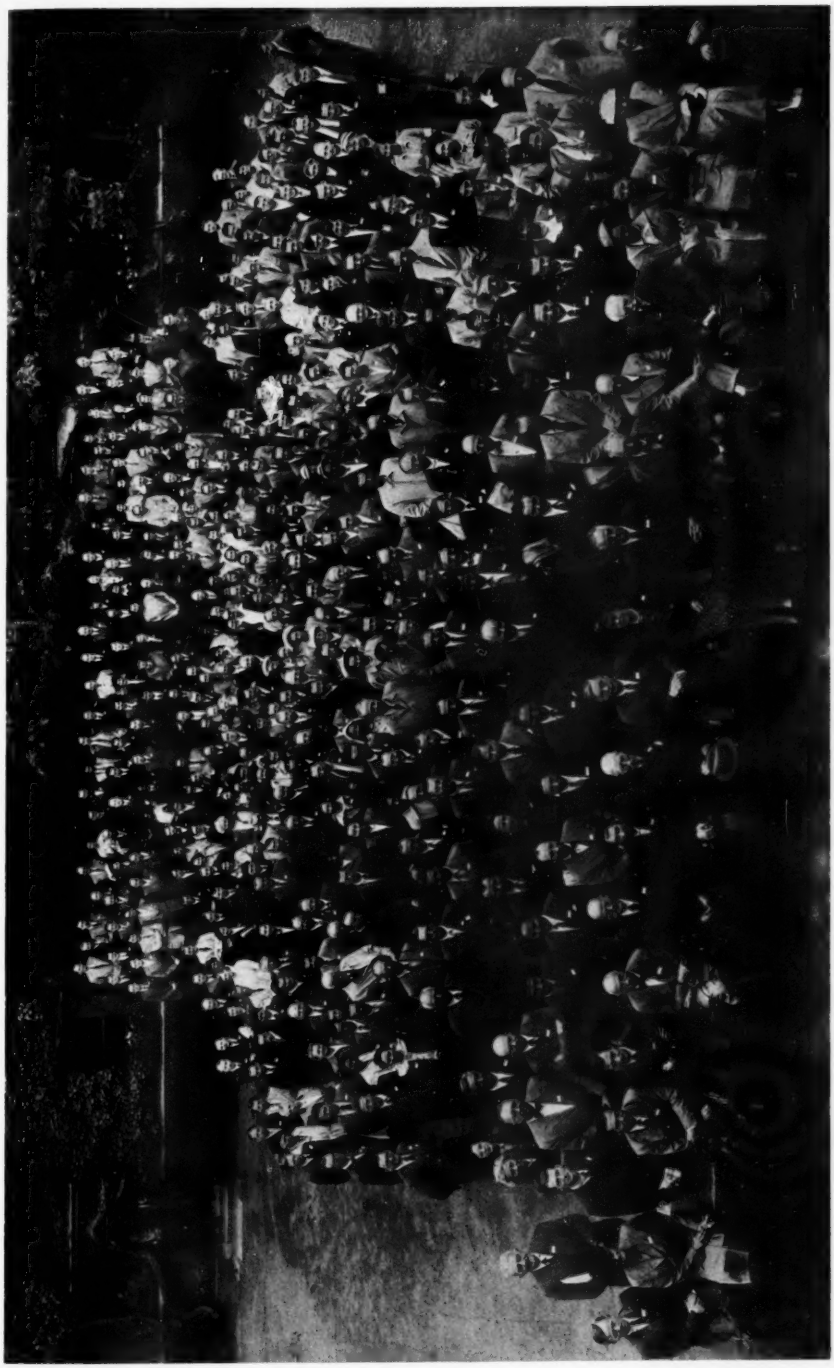
Little of value can be said about the question of the organization of entertainments and hospitality, since obviously local conditions must play a preponderant part in the preparation of this side of any Congress. The main difficulties were the complete uncertainty of numbers when booking tickets, etc., the necessarily limited accommodation for any specific form of entertainment, and the inevitability (in this country) of having to cater for both fine and wet weather. The general principles were to arrange for twice the probable number—this led to some financial losses—and to make use of free facilities wherever possible. For the benefit of future

organizers it may be stated that anatomists are not likely to want to play bowls. They are, however, passionately addicted to Stratford-on-Avon. It was suggested by many that it would have been desirable to arrange for booking for such trips as Stratford before the Congress assembled, so that a greater number could have been accommodated. Unfortunately this would have made no difference in this particular instance, since all booking had to be conducted through agents, and all the available seats were in fact booked at the outset—even before the acceptances to the Congress began to come in.

One might perhaps classify as entertainment the official photograph which was taken on the morning of the 25th; copies were available for members to order before the Congress dispersed on the 28th (see Pl. 1). In addition, several 'candid' and often distinctly entertaining photographs were taken by members of the staff of the Department of Anatomy at Oxford. These were posted daily at the Congress centre, where they could be ordered.

The one entertainment which can always be relied on (and is also free) is the privilege of talking anatomical shop with one's colleagues, and it was suggested that it would have been a good idea to arrange for the centre in Rhodes House to be open in the evenings to act as a sort of club where this pastime could be indulged in. It was unfortunate that this was not done, particularly since it proved impossible to arrange any mass dinner or other similar function owing to the lack of suitable accommodation in Oxford for the large numbers involved. It was for this reason that a dance was held in Oriel College on the last night of the Congress, as it was felt that it would afford members an opportunity of getting together informally.

These remarks on the preparations for the Congress are necessarily written from a somewhat personal angle, but nevertheless it is hoped that they may prove of interest and of some value to anyone whose duty it may become to organize a similar function in the future. Many members were kind enough to send in comments and suggestions both before and after the Congress. All these were very much appreciated, and so far as possible were acted upon. It is only by writing down the experiences gained and the shortcomings observed that a material basis can be provided on which to build subsequent Congresses.



SINCLAIR—Anatomical Congress, 1950: organization and logistics

APPENDIX 1

SLIPS ATTACHED TO THE INVITATION TO THE CONGRESS, INCLUDING VERSIONS MODIFIED ON THE BASIS OF EXPERIENCE

A

(This slip must reach the address below not later than 1 MARCH 1950)

I PROPOSE to attend the International Anatomical Congress, 1950, to be held in Oxford from Tuesday, 25 July, to Friday, 28 July 1950.

*I enclose herewith ..... value ..... made payable to 'Anatomical Congress'.

OR

I have instructed my bankers to pay the sum of ..... to Barclays Bank, Ltd., High Street, Oxford, for the credit of the account of the International Anatomical Congress, 1950, in payment of:

*(*a*) My subscription (£2.10*s.* 0*d.*)

(*b*) Registration Fee(s) for ..... associates (£1 each)

* Delete words that are not applicable

Name .....

(IN BLOCK LETTERS OR TYPEWRITTEN)

Address .....

.....

.....

To BARCLAYS BANK, LTD. (I.A.C. 1950), HIGH STREET, OXFORD, ENGLAND

B

(This slip must reach the address below not later than 1 MARCH 1950)

*I PROPOSE / I do not propose } to attend the International Anatomical Congress, 1950.

*1. I am making my own arrangements for accommodation.

2. Please reserve accommodation for me from the night of Monday, 24 July, to the afternoon of Friday, 28 July 1950, as follows:

*(*a*) In a hotel, if possible.

(*b*) In a College or College hostel.

(*c*) A double room in either of the above as my husband/wife is also attending the Congress as a full member/an associate.

3. Please reserve additional accommodation for ..... whom I have registered as associates.

* Delete words that are not applicable

Name ..... ♂ *or* ♀

(IN BLOCK LETTERS OR TYPEWRITTEN)

Address .....

.....

.....

To THE CONGRESS ORGANIZER, INTERNATIONAL ANATOMICAL CONGRESS, 1950, DEPARTMENT OF HUMAN ANATOMY, UNIVERSITY MUSEUM, OXFORD, ENGLAND

C

(This slip must reach the address below not later than 1 MARCH 1950)

I DESIRE to make a communication to the International Anatomical Congress, 1950, on the subject of:

.....

.....

An abstract of my communication, written in .....(language) and not exceeding 400 words,

*is enclosed

should reach you by 1 March 1950.

*I propose to illustrate my remarks by:

*Lantern Slides Size.....

Film Strip Size.....

Epidiascope (Material must not exceed 6 in. by 6 in.)

* Delete words that are not applicable

Name .....

(IN BLOCK LETTERS OR TYPEWRITTEN)

Address .....

.....

.....

To THE CONGRESS ORGANIZER, INTERNATIONAL ANATOMICAL CONGRESS, 1950, DEPARTMENT OF HUMAN ANATOMY, UNIVERSITY MUSEUM, OXFORD, ENGLAND

D

(This slip must reach the address below not later than 1 MARCH 1950)

I DESIRE to give a demonstration of:

.....

.....

to the International Anatomical Congress, 1950.

Details: .....

.....

.....

Name .....

(IN BLOCK LETTERS OR TYPEWRITTEN)

Address .....

.....

.....

To THE CONGRESS ORGANIZER, INTERNATIONAL ANATOMICAL CONGRESS, 1950, DEPARTMENT OF HUMAN ANATOMY, UNIVERSITY MUSEUM, OXFORD, ENGLAND

E

(This slip must reach the address below not later than 1 MARCH 1950)

I DESIRE to exhibit a cinematograph film (or films) to the International Anatomical Congress, 1950, on the subject of:

.....

.....

Details: Length of film ..... feet or metres

Width of film ..... mm.

Sound or Silent

Name .....

(IN BLOCK LETTERS OR TYPEWRITTEN)

Address .....

.....

.....

To THE CONGRESS ORGANIZER, INTERNATIONAL ANATOMICAL CONGRESS, 1950, DEPARTMENT OF HUMAN ANATOMY, UNIVERSITY MUSEUM, OXFORD, ENGLAND

F

(This slip must reach the address below not later than 1 MARCH 1950)

DURING the week before/the week after the International Anatomical Congress, 1950, I should like to visit:

Laboratory .....

Subject .....

OR

Laboratory .....

Subject .....

Name .....

(IN BLOCK LETTERS OR TYPEWRITTEN)

Address .....

.....

.....

To THE CONGRESS ORGANIZER, INTERNATIONAL ANATOMICAL CONGRESS, 1950, DEPARTMENT OF HUMAN ANATOMY, UNIVERSITY MUSEUM, OXFORD, ENGLAND

MODIFIED VERSION OF FORM C

(Unless this slip reaches the address below before.....the communication cannot be included in the printed programme.)

I (WE) desire to make a communication to the International Anatomical Congress.

TITLE: .....

An abstract of my (our) communication, written in the language in which it will be delivered, and not exceeding 400 words, is enclosed. (If possible, a translation should also be enclosed.)

I (WE) propose to illustrate my (our) remarks by:

Lantern slides (transparencies) Size.....(Lantern slides of the following sizes only can be shown:.....)

Film strip (35 mm. only)

Epidiascope (Material must not exceed 15 cm. × 15 cm.)

*Cinematograph film (16 mm. only) Length of film.....metres

Sound or Silent

I (WE) should like to show (apart from the above communication) a cinematograph film

TITLE: .....

Length of film.....metres

Sound or Silent

†*Name(s)*.....

(IN BLOCK LETTERS OR TYPEWRITTEN)

Address .....

.....

.....

* The total time available for communication plus film will not exceed.....minutes.

† In the case of joint papers the name of the member who is to read the paper should come first, and his collaborators need not fill in a separate form; their names will of course be included on the programme.

MODIFIED VERSION OF FORM D

(Unless this slip reaches the address below before.....the demonstration cannot be included in the printed programme.)

I (WE) desire to give a demonstration to the International Anatomical Congress.

TITLE: .....

REQUIREMENTS:

(1) Bench space.....metres

(2) Wall space .....square metres (For diagrams etc.)

(3) Microscopes

(*a*) Type.....

(*b*) Number ..... (No more than.....can be allotted to any one demonstrator)

(*c*) Lenses.....

(*d*) Lighting .....

(4) Museum jars, etc. .....

(5) Viewing boxes .....

(6) Electrical (plugs, sockets, etc.) ..... (The circuits available are.....)

(7) Special .....

Name(s).....

(IN BLOCK LETTERS OR TYPEWRITTEN)

Address .....

.....

.....

In the case of joint demonstrations the name of the senior member should come first, and his collaborators need not fill in separate forms; their names will of course be included on the programme.

APPENDIX 2

LIST OF MEMBERS

ABADIR, A. G. (Egypt)
ABADIR, F. M. (Alexandria, Egypt)
ABBIE, A. A. (Adelaide, Australia)
ABOULAFIA, E. D. (Geneva, Switzerland)
ADEY, W. R. (Adelaide, Australia)
AITKEN, J. T. (London, G.B.)
ALGIRE, G. H. (Bethesda, Md., U.S.A.)
ALLISON, A. C. (Oxford, G.B.)
AMOROSO, E. C. (London, G.B.)
AMPRINO, R. M. (Turin, Italy)
ANDREASSI, G. (Rome, Italy)
ANDREWS, W. H. H. (Liverpool, G.B.)
ANTÚNEZ, L. LÓPEZ (Mexico D.F., Mexico)
ARA, P. (Madrid, Spain)
ARIËNS KAPPERS, J. (Groningen, Holland)
ARMSTRONG, J. A. (London, G.B.)
ARSUFFI, ELENA (Zurich, Switzerland)
ASHLEY, G. M. (St Andrews, G.B.)
ASHURST, G. M. (St Andrews, G.B.)

BACSICH, P. (Glasgow, G.B.)
BALANKURA, K. (Cambridge, G.B.)
BALDUZZI, P. (Pavia, Italy)
BARADI, A. F. (London, G.B.)
BARER, GWENDA (Oxford, G.B.)
BARER, R. (Oxford, G.B.)
BARGMANN, W. (Kiel, Germany)
BARNETT, C. H. (London, G.B.)
BARR, M. L. (London, Ontario, Canada)
BAUD, A. (Geneva, Switzerland)
BAUER, K. F. (Erlangen, Germany)
BAUMANN, J. A. (Geneva, Switzerland)
BECCARI, N. (Florence, Italy)
BEJDL, W. (Vienna, Austria)
BÉLANGER, L-F. (Ottawa, Canada)
BELLAIRS, A. d'A. (London, G.B.)
BENNINGHOFF, A. (Marburg/Lahn, Germany)
BENOIT, J. (Strasbourg, France)
BERGMAN, R. A. M. (Amsterdam, Holland)
BIJTEL, J. HUBERTHA (Groningen, Holland)
BLACKSTAD, T. (Oslo, Norway)
BLOCKLEY, CECILLIE, M. B. (London, G.B.)
BORG, F. (Malta)
BORGHESE, E. (Pavia, Italy)
BORK-FELTKAMP, A. J. van (Amsterdam, Holland)
BÓSKOVIĆ, MARJAN (Belgrade, Yugoslavia)
BOURNE, G. (London, G.B.)
BOYCOTT, B. B. (London, G.B.)
BOYD, ELIZABETH (South Hadley, Mass., U.S.A.)
BOYD, G. I. (Leeds, G.B.)
BOYD, J. D. (London, G.B.)
BRAITHWAITE, J. L. (Manchester, G.B.)
BRANDER, J. (Galashiels, G.B.)
BRANDT, W. (Birmingham, G.B.)
BRASH, J. C. (Edinburgh, G.B.)
BRIFFA, J. (Malta)
BROOKS, C. Mc. C. (New York, N.Y., U.S.A.)
BRUSA, A. (Genoa, Italy)
BRUTO DA COSTA, V. V. P. (Lisbon, Portugal)
BUJARD, E. (Geneva, Switzerland)
BULL, M. M. (Cambridge, G.B.)
BURNS, R. K. (Baltimore, Md., U.S.A.)
BURRIDGE, W. (Oxford, G.B.)
BUTLER, H. (Cambridge, G.B.)

CAIRNS, H. W. B. (Oxford, G.B.)
CARLETON, ALICE (Oxford, G.B.)
CARMICHAEL, E. A. (London, G.B.)
CARRATO, A. (Salamanca, Spain)
CASTELNUOVO, GINA (Rome, Italy)
CATRITSIS, E. (Paris, France)
CAULLERY, M. (Paris, France)
CAUNA, N. (Newcastle-on-Tyne, G.B.)
CAUSEY, G. (London, G.B.)
CAVE, A. J. E. (London, G.B.)
CELESTINO DA COSTA, A. (Lisbon, Portugal)
CHACKO, LIZA (Vellore, S. India)
CHEN, J-M. (Cambridge, G.B.)
CHÊVREMONT, M. (Liége, Belgium)
CHIFFLET, J. (Paris, France)
CHINARD, F. P. (Baltimore, Md., U.S.A.)
CHIODI, V. (Bologna, Italy)
CHOUKÉ, K. S. (Philadelphia, Pa., U.S.A.)
CLARK, W. E. LE GROS (Oxford, G.B.)
COLE, MRS D. (Naro Moru, Kenya)
COLE, J. W. (Oxford, G.B.)
COLLIN, R. (Oxford, G.B.)
COLLINS, EMMA, C. (Ann Arbor, Mich., U.S.A.)
COLLISTER, Ruby M. (Leiden, Holland)
COOKE, Leonore S. (Ludhiana, Punjab, India)
CORNER, G. W. (Baltimore, Md., U.S.A.)
CRABB, E. D. (Boulder, Colorado, U.S.A.)

CRAWFORD, G. N. C. (Oxford, G.B.)
CREED (COOPER), Sybil (Oxford, G.B.)
CUMMINS, H. (New Orleans, La., U.S.A.)

DALCQ, A. (Brussels, Belgium)
DANIEL, P. M. (Oxford, G.B.)
DANKMEIJER, J. (Leiden, Holland)
DARCUS, H. D. (Oxford, G.B.)
DARCY, D. A. (Oxford, G.B.)
DAVIES, D. V. (London, G.B.)
DAVIES, F. (Sheffield, G.B.)
DAVIES, J. (Cambridge, G.B.)
DEBEYRE, J. (Paris, France)
DELATTRE, A. (Lille, France)
DELMAS, A. (Paris, France)
DESAIVE, P. (Liége, Belgium)
DESCLAUX, P. (Paris, France)
DETH, J. H. M. G. van (Utrecht, Holland)
DICKSON, Lilian M. (London, G.B.)
DORAN, F. S. A. (Manchester, G.B.)
DOW, D. R. (Dundee, G.B.)
DUBOIS, Anne Marie (Geneva, Switzerland)
DUCKWORTH, J. W. A. (Edinburgh, G.B.)
DURWARD, A. (Leeds, G.B.)

EAYERS, J. T. (Birmingham, G.B.)
ECKSTEIN, P. (Birmingham, G.B.)
EINARSON, L. (Aarhus, Denmark)
EL ASFOURY, Z. M. (London, G.B.)
EL BATRAWI, A. M. (Cairo, Egypt)
Lady ELLIOT SMITH (Oxford, G.B.)
ELLIS, A. R. (Singapore)
ENCINAS, E. (Lima, Peru)
ESCOLAR, J. (Granada, Spain)
EVANS, D. H. L. (London, G.B.)
EVANS, H. M. (Berkeley, Cal., U.S.A.)
EYRIES, C. (Paris, France)

FIELDING, Una (Alexandria, Egypt)
FIGGE, F. H. J. (Baltimore, Md., U.S.A.)
FILOGAMO, G. (Turin, Italy)
FLEXNER, L. B. (Baltimore, Md., U.S.A.)
FLOYD, W. F. (London, G.B.)
FORBES, T. R. (New Haven, Conn., U.S.A.)
FOX, C. A. (Milwaukee, Wis., U.S.A.)
FRANCIS, E. T. B. (Sheffield, G.B.)
FRANKLIN, K. J. (London, G.B.)
FRIANT, Madeleine (Paris, France)
FROLICH, M. (London, G.B.)
FYFE, F. W. (Aberdeen, G.B.)

GAILLARD, P. J. (Leiden, Holland)
GAIRNS, F. W. (Glasgow, G.B.)
GALLI, J. A. (Genoa, Italy)
GARDNER, W. U. (New Haven, Conn., U.S.A.)
GARRAUD, R. M. (Lille, France)
GARVEN, H. S. D. (Glasgow, G.B.)
GASIĆ, G. (Santiago, Chile)
GEOGHEGAN, B. (Oxford, G.B.)
GETZ, B. (Oslo, Norway)
GIROUD, A. (Paris, France)
GLEES, P. (Oxford, G.B.)
GLIMSTEDT, G. (Lund, Sweden)
GOLDBY, F. (London, G.B.)
GOTTLIEB, O. (Copenhagen, Denmark)
GREEN, J. D. (Detroit, Mich., U.S.A.)
GREEN, R. B. (Newcastle-on-Tyne, G.B.)
GREEN, S. H. (Birmingham, G.B.)
GRÉGOIRE, C. (Brussels, Belgium)
GREULICH, W. W. (Stanford, Cal., U.S.A.)
GRIFFITHS, V. G. (Malta)
GROSS, J. (Montreal, P.Q., Canada)

HAGEN, Emmi (Bonn, Germany)
HAINES, R. Wheeler (Cairo, Egypt)
HAMILTON, G. F. (Aberdeen, G.B.)
HAMILTON, W. J. (London, G.B.)
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HARKNESS, R. D. (Slough, G.B.)
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ROSSI, Franca (Genoa, Italy)
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THOMAS, P. K. (London, G.B.)
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VENERONI, G. (Pavia, Italy)
VERDUN, M. (Paris, France)
VIGLIANI, F. (Turin, Italy)
VIRNO, V. (Rome, Italy)
VIZOSO, A. D. (London, G.B.)
VONWILLER, P. (Rheinau, Switzerland)
VOSBURGH, G. J. (Baltimore, Md., U.S.A.)

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WALBERG, F. (Oslo, Norway)
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Walls, W. K. J. (Leeds, G.B.)
Walmsley, R. (St Andrews, G.B.)
Warwick, R. (Manchester, G.B.)
Weber, J. A. (Geneva, Switzerland)
Weddell, G. (Oxford, G.B.)
Weeks, Blanche (Dublin, Eire)
Weiner, J. S. (Oxford, G.B.)
Wendt, G. G. (Marburg/Lahn, Germany)
West, C. M. (Cardiff, G.B.)
Whitney, R. J. (Oxford, G.B.)
Wilde, F. R. (London, G.B.)
Wilson, Katharine F. (Dublin, Eire)
Winckler, G. (Strasbourg, France)
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Yang, S. H. (Kirkhill, G.B.)
Yoffey, J. M. (Bristol, G.B.)
Young, A. (Glasgow, G.B.)
Young, J. Z. (London, G.B.)

Zander, E. F. (Oxford, G.B.)
Zeren, Z. (Istanbul, Turkey)
Zorzoli, G. (Pavia, Italy)
Zuckerman, S. (Birmingham, G.B.)

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Acosta, E. (Mexico, D.F., Mexico)
Ayer, A. A. (Madras, India)

Baxter, J. S. (Bristol, G.B.)
Belou, P. (Buenos Aires, Argentina)
Berg, K. (Oslo, Norway)
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Brodal, A. (Oslo, Norway)

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Di Dio, L. J. A. (S. Paulo, Brazil)

Erhart, E. A. (S. Paulo, Brazil)
Ettinger, G. H. (Ottawa, Ontario, Canada)

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Huijbers, Margot M. (Amsterdam, Holland)

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Koenigswald, G. H. R. von (Utrecht, Holland)
Krause, W. (Vienna, Austria)

Landau, E. (Lausanne, Switzerland)
Locchi, R. (S. Paulo, Brazil)

Macklin, Madge T. (Columbus, Ohio, U.S.A.)
Mijsberg, W. A. (Utrecht, Holland)
Mitchinson, A. G. H. (Cardiff, G.B.)
Mosinger, M. (Coimbra, Portugal)

Saunders, R. L. de C. H. (Halifax, W.S., Canada)
Schaepelynck, J. (Beirut, Lebanon)
Scharrer, Berta (Denver, Colorado, U.S.A.)
Scharrer, E. A. (Denver, Colorado, U.S.A.)
Stahl, A. (Marseilles, France)
Stieve, H. (Berlin, Germany)
Straus, W. L. (Baltimore, Md., U.S.A.)

Tondury, G. (Zurich, Switzerland)

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Brandt, Mrs W. (Great Britain)
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Bujard, Miss (Geneva, Switzerland)
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GIRAN, Mrs Claire (Paris, France)
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LORENTZEN, Mrs K. A. (Risskov, Denmark)
LUND, Mrs T. (Aarhus, Denmark)
LÜTKEN, Mrs P. (Aalborg, Denmark)

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MARTIN, C. P. (Montreal, Canada)
MENSCHIK, Mrs Z. (Ottawa, Canada)
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MORUJO, Mrs A. Alvarez (Salamanca, Spain)

NAIR, Mrs S. R. (Bombay, India)

ODAR, Mrs I. (Ankara, Turkey)
O'RAHILLY, Mrs R. (Great Britain)

PALUMBI, Mrs G. (Pavia, Italy)
PATTEN, Mrs B. M. (Ann Arbor, U.S.A.)
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PETREN, Mrs T. (Stockholm, Sweden)

RAYNAUD, Mrs A. (Paris, France)
ROBLIN, Miss Bluette (Geneva, Switzerland)
RONDELLI, Miss Lia (Turin, Italy)
ROSSI, Mrs F. (Genoa, Italy)

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SOULAIRAC, Mr (Paris, France)
STONE, Mrs L. S. (New Haven, U.S.A.)
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WOERDEMAN, Mrs M. W. (Amsterdam, Holland)
WYKE, Mrs B. D. (Great Britain)

APPENDIX 3

LIST OF COMMUNICATIONS

General Anatomy

ACOSTA, E. (Mexico). Clasificación funcional de los seños de la duramadre.

AYER, A. A. and MARIAPPA, D. (Madras, India). A radiographic study of ossification in the foetal Indian elephant.

BAUER, K. F. (Erlangen, Bavaria). Versuche mit Metallbeschattung menschlicher Gewebe.

BAZETT, H. C. and ROBINSON, S. (Philadelphia, U.S.A.). A theory of reflex control of temperature at rest and during exercise.

BEJDL, W. (Vienna, Austria). Effects of ultrasound on blood with regard to therapeutic application.

BELLAIRS, A. d'A. and UNDERWOOD, G. L. (London, G.B., and Jamaica, B.W.I.). Anatomical evidence bearing on the origin of snakes.

BELOU, P. (Buenos Aires, Argentina). Síntesis de la revisión realizada durante 33 años sobre la morfología del sistema arterial humano.

BRUTO DA COSTA, V. V. P. (Lisbon, Portugal). Musculus sternalis.

BURRIDGE, W. (Oxford, G.B.). Climatic adaptation in frog's muscle.

BURRIDGE, W. (Oxford, G.B.). The make-up of the frog's ventricle.

BUTLER, H. (Cambridge, G.B.). Veins of the oesophagus in man.

CATRITSIS, E. and JUSTER, M. (Paris, France). Sur quelques facteurs pouvant déterminer la valeur fonctionnelle des artères axillaire et humérale.

CHIODI, V. (Bologna, Italy). New observations on the conduction apparatus in the heart of the pig (*Sus scrofa*).

COLLISTER, Ruby M., DANKMEIJER, J. and SNELLEN, H. A. (Leiden, Holland). Illustrations of cardiac malformations with radiology.

DAVIES, F., FRANCIS, E. T. B. and KING, T. S. (Sheffield, G.B.). The conducting (connecting) system of the heart of *Alligator mississippiensis*.

DELMAS, A. and BERTRAND, G. (Paris, France). The parietal vein.

DELMAS, A. and CHIFFLET, J. (Paris, France). The dural venous lakes of the vertex.

EYRIES, C., ABOULKER, P. and GANDON. (Paris, France). Sur le nerf facial extra-petreux intra-parotidien.

FIGGE, F. H. J. (Baltimore, U.S.A.). Anatomical studies on the attempts to minimize the pain of parenteral therapy by jet injection.

FLOYD, W. F. and SILVER, P. H. S. (London, G.B.). Activity in the trunk, thigh, and leg muscles during standing in man: an electromyographic study.

GREULICH, W. W. (Stanford, U.S.A.). The value of radiographs of the hand and wrist in determining the developmental status of children.

GUARDABASSI, A. and SACERDOTE, Maria (Turin, Italy). New structural data on the integument of the Anura.

HAINES, R. W. (Cairo, Egypt). The mechanism of the interphalangeal joints.

HJORTSJÖ, C.-H. (Lund, Sweden). The topography of the intrahepatic duct system.

JOSEPH, J. (London, G.B.). The human thumb.

JUSTER, M. and CATRITSIS, E. (Paris, France). Données sur le calibre des artères axillaire et humérale chez l'homme.

KAHNT, Ursula and DANKMEIJER, J. (Leiden, Holland). Topography of the pulmonary veins.

KEMP, F. H. (Oxford, G.B.). The mechanism of deglutition.

KUHN, H. (Paris, France). The development of the film as a basic teaching aid in Anatomy.

LATARJET, M. and JUTTIN, P. (Lyon, France). La distribution périphérique des artères bronchiques.

MacCONAILL, M. A. (Cork, Ireland). The mechanical structure of articulating cartilage.

MACHADO DE SOUSA, O. (S. Paulo, Brazil). Sur les points faibles de la région lombaire chez les nègres brésiliens.

MAROUN, Th., SCHAEPELYNCK, J., BADARO and GUERGUERIAN, S. (Beirut, Lebanon). Absence congénitale d'appendice: considérations anatomiques et pathologiques.

MENSCHIK, Z. (Ottawa, Canada). Nutritional changes in interscapular adipose tissue in guinea-pigs, with special reference to vitamin E.

MONTEIRO, H. (Porto, Portugal). L'ancienne technique de préparation des os. À propos des lettrines de la Fabrica de Vésale.

MORUJO, A. Alvarez (Salamanca, Spain). Variaciones estructurales en las ramas de las arterias.

PAPAMILTIADES, M. (Athens, Greece). Les lymphatiques de l'artère pulmonaire de l'homme.

PEACOCK, A. (London, G.B.). Observations on the pre- and post-natal human intervertebral disc.

RAWLINSON, H. E. (Edmonton, Canada). The relation between the mammary gland and apocrine sweat glands.

ROLLHÄUSER, H. (Marburg/Lahn, Germany). Die Festigkeit menschlicher Sehnen in Abhängigkeit von Lebensalter und Konstitution.

SCHAEPELYNCK, J., CIAUDO, D. and SERHAL, F. (Beirut, Lebanon). Anatomie chirurgicale du nerf auriculo-temporal.

SMITH, J. W. (St Andrews, G.B.). The elastic properties of the anterior cruciate ligament of the rabbit.

VASSAL, P. A. (Paris, France). Sur quelques asymétries chez l'homme.

WENDT, G. G. (Marburg/Lahn, Germany). Einfluss normaler Flüssigkeitsaufnahme auf den Wassergehalt des Muskels.

WINCKLER, G. (Strasbourg, France). Aspects évolutifs du muscle grand oblique de l'abdomen (external oblique).

WRIGHT, G. H. (Cambridge, G.B.). The reaction-time to warmth.

ZEREN, Z. (Istanbul, Turkey). Un cas très rare de trajet du nerf phrénique à travers les parois de la veine sous-clavière.

Neurology

ABBIE, A. A. and ADEY, W. R. (Adelaide, Australia). Motor co-ordination mechanisms in the central nervous system.

ADEY, W. R. (Adelaide, Australia). The giant nerve fibre systems of an Australian earthworm of the genus *Megascolex*.

ALLISON, A. C. (Oxford, G.B.). On the connexions of the primary olfactory cortex.

ARIËNS KAPPERS, J. (Groningen, Holland). Meninges and cerebro-spinal fluid circulation in *Amblystoma mexicanum*.

BARGMANN, W. (Kiel, Germany). Über das hypothalamo-hypophysäre System.

BARR, M. L. (London, Canada). The morphology of the nerve cell nucleus, with particular reference to differences according to sex.

BENNINGHOFF, A. (Marburg/Lahn, Germany). Funktionelle Hypertrophie von Nervenzellen.

BERG, K. (Oslo, Norway). The toxic effect of streptomycin on the eighth cranial nerve.

Brodal, A., Walberg, F. and Blackstad, T. (Oslo, Norway). Termination of spinal afferents to the inferior olive in the cat.

Carrato, A. (Salamanca, Spain). The variations of the Nissl pattern in the nervous cells of cerebral fragments cultivated *in vitro*.

Chacko, Liza. (Vellore, India). Analysis of fibre-size of the optic nerve in mammals.

Clark, W. E. Le Gros (Oxford, G.B.). The projection of the olfactory area of the nasal cavity on the olfactory bulb.

Collin, R. (Oxford, G.B.). The relation between nerve supply and hair follicle size in the skin of the rat.

Davies, F., Francis, E. T. B. and King, T. S. (Sheffield, G.B.). Nerve cells in the ventricles of the hearts of mammals.

Escolar, J. (Granada, Spain). The afferent connexions of the apical cochlear fibres in the cat. An analysis by Marchi and Rasdolsky methods.

Evans, D. H. L. and Vizoso, A. D. (London, G.B.). The ratio of myelin to axon and the determination of fibre diameter during the development of peripheral nerves.

Flexner, L. B. and Flexner, J. B. (Baltimore, U.S.A.). Biochemical and functional differentiation of nerve cells in the frontal cerebral cortex of the foetal guinea-pig.

Fox, C. A., Ubeda-Purkis, M. and Massopust, L. C. (Milwaukee, U.S.A.). Structure of the cerebellar cortex in the adult monkey (*Macaca mulatta*). A Golgi study.

Gambarin, A. (London, G.B.). Histogenesis and cytogenesis of microglia and its distribution in the nervous system.

Garven, H. S. D. and Gairns, F. W. (Glasgow, G.B.). The sympathetic ground plexus.

Giroud, A. and Boisselot, J. (Paris, France). Perturbations dans le développement du système nerveux à la suite de carences.

Glees, P. and Bailey, R. (Oxford, G.B.). Course and termination of the spino-thalamic tract and the medial fillet in man.

Glees, P., Cole, J., Whitty, C. W. M. and Cairns, H. (Oxford, G.B.). The effects of lesions in the cingulate gyrus and adjacent areas in monkeys.

Glees, P., Liddell, E. G. T. and Phillips, C. G. (Oxford, G.B.). The course of the medial fillet in the lower brain stem of the cat.

Harkmark, W. (Oslo, Norway). The development of the nuclei pontis and the inferior olive in the chick.

Ingram, W. R., Knott, J. R., Wheatley, M. D. and Summers, T. B. (Iowa, U.S.A.). Physiological relationships between hypothalamus and cerebral cortex.

Jordão Pereira, C. M. (Lisbon, Portugal). Acêrca de alguns problemas do sistema neuro-vegetativo.

Krabbe, K. H. (Copenhagen, Denmark). Comparative embryology and anatomy of the pineal body in reptiles, birds, and mammals.

Krmpotić, J. (Zagreb, Yugoslavia). Observations concerning the spiral lobules of the human cerebellum.

Kuhlenbeck, H. (Philadelphia, U.S.A.). Homology in the thalamus dorsalis of mammals and submammalian amniota.

Landau, E. (Lausanne, Switzerland). Neurohistology of the heart.

Levi, G. (Turin, Italy). Nombre et volume des neurones dans deux espèces de Caviides de taille différente.

Lewy, R. B. (Chicago, U.S.A.). Anatomical and clinical evaluation of head pain attributed to the sphenopalatine ganglion.

Machado de Sousa, O. (S. Paulo, Brazil). Les nerfs splanchniques chez les nègres brésiliens.

Mazzucchelli, B. (Pavia, Italy). Sulla innervazione della epifisi dei mammiferi.

Meyer, Margaret (Oxford, G.B.). An experimental study of fronto-hypothalamic connexions in the monkey.

Mitchell, G. A. G. (Manchester, G.B.). The intrinsic innervation of the kidneys.

Monro, P. A. G. (London, G.B.). Observations on sympathetic pathways after paravertebral sympathectomies.

MORUJO, A. Alvarez (Salamanca, Spain). Cambios morfológicos de las células del plexo mientérico en actividad funcional.

NAPIER, J. R. and BOWDEN, Ruth E. M. (London, G.B.). The return of cutaneous sensibility to full-thickness skin flaps in man.

NAUTA, W. J. H. (Zurich, Switzerland). Some connexions of the septal area of the forebrain in the rat.

NOBACK, G. J. and Anderson, F.D. (San Juan, Puerto Rico). Further studies of the structure of the human ductus arteriosus, with special reference to nerves and nerve endings in its wall.

PALUMBI, G. (Pavia, Italy). A new nervous apparatus of receptive type in the cochlea of the human ear.

PEARSON, A. A. (Portland, U.S.A.). The communicating rami of the sympathetic trunk in the cervical and thoracic levels in the human foetus.

PETERS, Virginia B. (Baltimore, U.S.A.). Studies on the morphological differentiation of nerve cells in the frontal cerebral cortex of the foetal guinea-pig.

PUENTE, J. (Salamanca, Spain). Terminaisons nerveuses dans le muscle diaphragme.

QUILLIAM, T. A. (London, G.B.). Diameter variations of nerve fibres along normal and regenerating sensory nerve trunks.

SANDERS, F. K. (Oxford, G.B.). Neuronal transport of the virus of mouse encephalomyelitis.

SANDERS, F. K. and MACDONALD, F. M. E. (Oxford, G.B.). Factors affecting the susceptibility of mice to the virus of mouse encephalomyelitis.

SCHARRER, E. A. (Denver, U.S.A.). The significance of cerebral capillary patterns.

SILVA PINTO, M. da and MONTEIRO, H. (Porto, Portugal). L'innervation sympathique des vaisseaux lymphatiques.

SINCLAIR, D. C. and HINSHAW, J. R. (Oxford, G.B.). Fibre size and cutaneous sensibility.

SOULAIRAC, A. and DESCLAUX, P. (Paris, France). Aspects nerveux centraux de certains processus d'agression.

SPRAGUE, J. M. and MEYER, Margaret (Oxford, G.B.). An experimental study of the fornix in the rabbit.

STONE, L. S. (New Haven, U.S.A.). The development of normal and abnormal vision in transplanted eyes.

THOMAS, P. K. (London, G.B.). The development of the corpus callosum and its influence on the hippocampus.

THOMPSON, I. M. (Winnipeg, Canada). The terminology of visceral innervation.

TOMASCH, J. (Vienna, Australia). Elektronenoptische Untersuchungen der Endoneuralscheide.

VONWILLER, P. (Rheinau, Switzerland). New staining methods for the nervous system.

WALBERG, F. (Oslo, Norway). The lateral reticular nucleus of the medulla oblongata. A comparative anatomical study.

WARWICK, R. (Manchester, G.B.). The internal topography of the oculomotor nucleus in the rhesus monkey.

WILDE, F. R. (London, G.B.). Perivascular neural patterns in the femoral region.

WINDLE, W. F. and CHAMBERS, W. W. (Philadelphia, U.S.A.). Spinal cord regeneration associated with a cellular reaction induced by administration of a purified bacterial pyrogen.

ZANDER, E. and GLEES, P. (Oxford, G.B.). Course and termination of the tegmento-olivary tract studied in two cases of midbrain tractotomy in man.

ZORZOLI, G. (Pavia, Italy). Ricerche e considerazioni su di alcuni pigmenti cromolipoidi.

ZORZOLI, G. and MAGGI, G. (Pavia, Italy). Contributo allo studio del parasimpatico spinale.

Histology

AMPRINO, R. (Turin, Italy). Bone reconstruction.

ANDREASSI, G. (Rome, Italy). The polarity of the outer cells of the enamel organ and particularly of the ameloblasts.

BARER, Gwenda R. (Oxford, G.B.). Radiography of lymphatics.

BAUD, A. (Geneva, Switzerland). Ultrastructure of the nuclear membrane.

BÉLANGER, L.-F. (Ottawa, Canada) and LEBLOND, C. P. (Montreal, Canada). Autographic visualization of the mechanism of bone and tooth formation.

BENOIT, J. and CLAVERT, J. (Strasbourg, France). Action de la folliculine sur la calcémie et l'ossification médullaire chez les oiseaux.

BRASH, J. C. (Edinburgh, G.B.). Growth mechanics of the skull.

BRUSA, A. (Genoa, Italy). Microscopic and auto-histo-radiographic studies of animals treated with uranium salts.

CRAWFORD, G. N. C. and BARER, R. (Oxford, G.B.). The action of simple fixatives on living cells observed with phase contrast microscopy.

CRAWFORD, G. N. C. and SANDERS, F. K. (Oxford, G.B.). The growth of *Vaccinia* virus in tissue cultures of adult rabbit skin epithelium.

DARCY, D. A. (Oxford, G.B.). The role of plasma cells in the breakdown of tissue homografts.

DUCKWORTH, J. W. A. (Edinburgh, G.B.). Observations on the coiled arteries of the renal sinus in man.

EINARSON, L. (Aarhus, Denmark). The progressive selective staining by gallocyanin-chromalum.

GRÉGOIRE, C. (Brussels, Belgium). Further studies on insect blood by phase contrast microscopy.

HANCOX, N. M. (Liverpool, G.B.). Motility of osteoclasts and its significance in bone absorption.

HARTROFT, W. S. (Toronto, Canada). 'Lipodiastaemata' (fatty cysts): their concept, structure, and fate in the liver of rats in experimental dietary cirrhosis.

HAYEK, H. von. (Wurzburg, Germany). The human alveolar epithelium and its functional changes.

HEGGIE, J. F. (London, G.B.). An assessment of the renal glomerular capillary velocity in the rabbit.

HOERR, N. L. (Cleveland, U.S.A.). The intermediary circulation of the mouse spleen, and its reactions to thermal changes.

HUTCHISON, J. (Glasgow, G.B.). Observations on bone transplantation.

JACOBSON, W. and WEBB, M. (Cambridge, G.B.). The two types of nucleic acid during mitosis.

JACOBY, F. and MARTIN, B. F. (Cardiff, G.B.). Biochemical and histochemical studies of alkaline phosphatase in normal guinea-pigs and in guinea-pigs after ligation of the common bile duct.

KRUSZYŃSKI, J. (Liverpool, G.B.). Some observations with the phase contrast microscope upon incinerated frog blood cells.

LANDBOE-CHRISTENSEN, E. (Copenhagen, Denmark). The argentaffine cells in the fundus and pylorus of man and the pig.

LUTKEN, P. (Aalborg, Denmark). Investigations on the morphogenesis of skeletal epiphyses.

MACKLIN, C. C. (London, Canada). The dust cells of the albino mouse lung.

MAEGRAITH, B. G. and ANDREWS, W. H. H. (Liverpool, G.B.). Micro-circulation in the liver.

PHILPOT, J. St L. (Harwell, G.B.). Theoretical comparison of methods of phase microscopy.

VAN DER SCHUEREN, G. (Louvain, Belgium). Anatomo-radiological study of the hepatic circulation.

YANG, S. H. and GOODALL, A. M. (Kirkhill, G.B.). Histological and histochemical studies on bovine 'sweat glands'.

YOUNG, A. (Glasgow, G.B.). Studies on the vascular architecture of the rat uterus.

Physical Anthropology

ALLISON, A. C., ROBERTS, D. F. and WEINER, J. S. (Oxford, G.B.). Somatotyping in its application to the negro physique.

ASHTON, E. H. and ZUCKERMAN, S. (Birmingham, G.B.). The influence of isolation on speciation in monkeys.

CLARK, W. E. LE GROS (Oxford, G.B.). Certain considerations of the Australopithecinae.

CLARK, W. E. LE GROS (Oxford, G.B.). Report on the fossil hominoid material collected by the British-Kenya Miocene Expedition in 1949.

DELATTRE, A. (Lille, France). Le mécanisme de la transformation du crâne animal en crâne humain.

FRIANT, Madeleine (Paris, France). Temporary dentition of man and anthropoid apes.

HENCKEL, C. (Concepción, Chile). Estudios somatológicos en indios mapuches de la provincia de Cautin (Chile).

KOENIGSWALD, G. H. R. von (Utrecht, Holland). Early human types from the Lower Pleistocene of Java.

LAMMERS, H. J. (Leiden, Holland). The physical anthropology of the population of East-Dawan (Central Timor, Indonesia), from data collected by Prof. Dr B. A. G. Vroklage, S.V.D.

LEAKEY, L. S. B. (Nairobi, Kenya). Evidence bearing on the environment of the Early Miocene apes in East Africa.

MACKLIN, Madge T. (Columbus, Canada). The use of monozygous and dizygous twins in the study of hereditary traits in man.

OAKLEY, K. P. (London, G.B.). Dating of fossil human remains in the light of fluorine and related data.

PESONEN, N. (Helsinki, Finland). On the racial elements of the Finns.

STRAUS, W. L. (Jr.) (Baltimore, U.S.A.). Man and the gibbons.

TORGERSEN, J. (Oslo, Norway). The developmental genetics and evolutionary significance of the metopic suture.

TROTTER, Mildred and DUGGINS, O. H. (St Louis, U.S.A.). Age changes in head hair of children.

VERDUN, M. (Paris, France). Étude morpho-biométrique de la tête humaine sur le sujet vivant.

WEINER, J. S. (Oxford, G.B.). Climatic adaptation and human phylogeny.

ZUCKERMAN, S. (Birmingham, G.B.). Taxonomy and human evolution.

Embryology and Morphogenesis

BALANKURA, K. (Cambridge, G.B.). The development of the thoracic duct in the sheep.

BAXTER, J. S. (Bristol, G.B.). Alkaline phosphatase in primordial germ cells in a 10 mm. human embryo.

BIJTEL, J. Hubertha (Groningen, Holland). The mode of outgrowth of the tail in amphibian larvae.

BORGHESE, E., PERRI, G. C. and GELMETTI, L. (Pavia, Italy). Culture *in vitro* di megaloblasti embrionali di pollo.

BOURNE, G. H. (London, G.B.). The effect of injury to a bone on the phosphorus metabolism of the skeleton.

BOYD, J. D. (London, G.B.). Argentaffin dendritic cells in the epithelium of the external auditory meatus and of the tympanic membrane of human embryos.

BOYD, J. D. and HAMILTON, W. J. (London, G.B.). Development of the placenta.

CHATTERJEE, H. (Calcutta, India). Formulation of lacrimal sacs and canaliculi in cyclops.

CORNER, G. W. and BARTLEMEZ, G. W. (Baltimore, U.S.A. and Chicago, U.S.A.). Intrinsic and environmental factors in abnormality of early primate embryos.

DALCQ, A. M. (Brussels, Belgium). The morphogenetic organization of the oocyte and follicle in some mammals, including man.

DAVIES, J. (Cambridge, G.B.). A study of the mesonephric tubules of the sheep and rabbit, with special reference to 'secretory' activity.

DESAIVE, P. (Liége, Belgium). Atrésie folliculaire par rupture intra-ovarienne chez les mammifères.

GAILLARD, P. J. (Leiden, Holland). *In vitro* experiments on the origin of female sex cells in man.

GREEN, S. H. and ZUCKERMAN, S. (Birmingham, G.B.). The follicle-spectrum of the ovary of the rhesus monkey.

HAMILTON, W. J. and HARRISON, R. J. (London, G.B.). Follicular growth and atresia during the ovarian cycle in the ferret.

HARKMARK, W. and GRAHAM, K. (Oslo, Norway). A modification of the Hillemann's method for closing eggs, and a method for operating on chick embryos after the amnion formation.

HUGGETT, A. St G. (London, G.B.). Factors concerned in placental permeability.

HUIJBERS, Margot M. (Amsterdam, Holland). The effects of castration of the chick embryo.

KRAUSE, W. (Vienna, Austria). The continuity of germ-layers in a human embryonal disk of 0·32 mm.

MIJSBERG, W. A. (Utrecht, Holland). The genesis of symmetrical double monsters in man.

MOLL, J. (Groningen, Holland). Ontogenetic development of the aperturae laterales ventriculi IV (Luschka) and of the apertura mediana (Magendie).

MORICARD, R. (Paris, France). Hormones, meiosis, and fertilization in mammals.

MULNARD, J. (Brussels, Belgium). Peculiarities of the nuclear behaviour during the oogenesis in *Acanthoscelides obtectus* S. (Coleoptera).

ODÉ, E. (Leiden, Holland). Development of the ductus arteriosus in the albino rat.

O'RAHILLY, R. (Sheffield, G.B.). The early development of synovial joints.

PATTEN, B. M. (Ann Arbor, U.S.A.). Beat propagation and valvular action in the embryonic heart.

RAYNAUD, A. (Paris, France). Recherches expérimentales sur le développement embryonnaire de la glande mammaire de la souris.

ROSSI, F., PESCETTO, G. and REALE, E. (Genoa, Italy). Histological studies on the phosphatase in the human embryo.

RUGH, R. (New York, U.S.A.). The effect of I^{131} on the nursing mouse, when administered to the mother.

SCHARRER, Berta V. (Denver, U.S.A.). The prothoracic glands of insects, an analogue of the thymus.

STIEVE, H. (Berlin, Germany). Neue Beobachtungen über die Ovulation des Menschen.

VOSBURGH, G. J. (Baltimore, U.S.A.). The source of foetal iron and the permeability of the guinea-pig placenta to iron as determined with radio-active iron.

WARD-ORSINI, Margaret C. and STRAUSS, F. (Madison, U.S.A.). A preliminary report on the time and place of fertilization of the mammalian egg.

WEST, C. M. (Cardiff, G.B.). A well-preserved human embryo of the primitive streak stage.

WINDLE, W. F. (Philadelphia, U.S.A.). The time of closure of the ductus arteriosus.

Endocrinology

ASHFORD, C. A., ROBINSON, F. B. and YOFFEY, J. M. (Bristol, G.B.). Changes in the 'loaded' suprarenal of the adult male rat in response to cold, adrenaline tartrate, and noradrenaline.

BRANDER, J. (Edinburgh, G.B.). Concerning the application of the term 'pars tuberalis'.

BRANDER, J. (Edinburgh, G.B.). A note on the arterial blood-supply of the human hypophysis.

CELESTINO DA COSTA, A. (Lisbon, Portugal). Nouvelles recherches sur l'histogénèse et la cytogénèse du cortex surrénal.

DESCLAUX, P. and SOULAIRAC, A. (Paris, France). La valeur histophysiologique de la cellule hypophysaire de castration.

DETH, J. H. M. G. van (Utrecht, Holland). On the influence of the hypophysis on sex determination in the avian embryo.

FORBES, T. R. (New Haven, U.S.A.). Pre-ovulatory production of progesterone.

GREEN, J. D. (Detroit, U.S.A.). The innervation and vascularization of the pituitary body.

HARRIS, G. W. (Cambridge, G.B.). The regenerative capacity of the hypophysial portal vessels.

HERLANT, M. (Brussels, Belgium). Action des corticoïdes sur la structure histologique du rein.

LEBLOND, C. P. and GROSS, J. (Montreal, Canada). Formation and fate of the thyroid hormone, thyroxine.

METCALF, W. K., NAIRN, Valerie N. and YOFFEY, J. M. (Bristol, G.B.). Effects of pituitary adrenotrophic hormone, and extract of suprarenal cortex on the bone marrow of the guinea-pig.

MILINE, R. (Sarajevo, Yugoslavia). L'effet du bruit et du tremblement sur le système neuroendocrinien.

MOSINGER, M. (Coimbra, Portugal). Anatomical pathology of aggressology; the hypothalamo-hypophysial system and neuro-endocrinology.

OETTLÉ, A. G. and HARRISON, R. G. (Oxford, G.B.). The effect of ischaemia, temporary and permanent, on the histological appearance of the testis of the adult rat.

SIMPSON, Miriam E., EVANS, H. M. and CHOH HAO LI (Berkeley, U.S.A.). Effect of pure pituitary follicle stimulating hormone (FSH) alone or in combination with chorionic gonadotropin (HCG) in hypophysectomized male rats.

SOULAIRAC, A. and DESCLAUX, P. (Paris, France). Les phosphatases du tractus génital des mammifères et leur déterminisme hormonal.

TAVARES DE SOUSA, A. (Coimbra, Portugal). La colloïde hypophysaire, son origine et sa signification.

WYBURN, G. M. and BACSICH, P. (Glasgow, G.B.). Some observations on the effect of stilboestrol administration on the adrenals in the guinea-pig.

APPENDIX 4

LIST OF DEMONSTRATIONS AND FILMS

DEMONSTRATIONS

Methods of microscopy

BARER, R. (Oxford, G.B.). New methods of microscopy; apparatus for micromanipulation and microphotometry.

GRÉGOIRE, C. (Brussels, Belgium). Phase-contrast micrographs of insect blood.

MITCHELL, G. A. G. (Manchester, G.B.). Microradiography of various tissues.

PHILPOT, J. St L. (Oxford, G.B.). The 'Spirocyclic' microscope.

Physical Anthropology

CLARK, W. E. LE GROS and LEAKEY, L. S. B. (Oxford, G.B. and Nairobi, Kenya). Early Miocene fossil apes from East Africa.

HARPER, W. F. (St Andrew, Jamaica, B.W.I.). Aboriginal skeletal remains from Jamaica.

KOENIGSWALD, G. H. R. von (Utrecht, Holland). Pithecanthropus and Meganthropus finds from the Lower Pleistocene of Java. Teeth of Gigantopithecus from southern China.

VERDUN, M. (Paris, France). An anthropometrical apparatus.

Neurology

Allison, A. C. (Oxford, G.B.). Olfactory connexions in Mammalia; experimental studies.

Barr, M. L. (London, Canada). Sex differences in the morphology of the nerve cell nucleus.

Baumann, J.-A. (Geneva, Switzerland). Innervation végétative des muscles striés.

Boyd, J. D. and Monro, P. A. G. (London, G.B.). Intermediate sympathetic ganglia and alternative sympathetic pathways to the limbs.

Cooper, S. and Daniel, P. M. (Oxford, G.B.). Nerve endings, including muscle spindles, in mammalian extrinsic eye muscles.

El Asfoury, Z. M. (London, G.B.). The innervation of the kidney.

Escolar, J. (Granada, Spain). A system of endocranial human topography according to the stereotaxic Horsley Clarke procedure.

Gairns, F. W. (Glasgow, G.B.). The innervation of the human gum.

Garven, H. S. D. and Gairns, F. W. (Glasgow, G.B.). The sympathetic ground plexus.

Glees, P. and Bailey, R. (Oxford, G.B.). The course and termination of the spinothalamic tract and the medial fillet in man.

Hagen, Emmi (Bonn, Germany). The microscopical anatomy of the hypophysis.

Hagen, Emmi (Bonn, Germany). The microscopical innervation of the kidney.

Krmpotić, J. (Zagreb, Yugoslavia). The shape, varieties, and development of the spiral lobules of the cerebellum.

Landau, E. (Lausanne, Switzerland). The neuro-histology of the heart.

Mazzucchelli, B. (Pavia, Italy). The structure and nerve supply of the epiphysis.

Meyer, Margaret (Oxford, G.B.). An experimental study of cortico-hypothalamic connections in the monkey.

Millen, J. W. (Cambridge, G.B.). The innervation of blood-vessels.

Nauta, W. J. H. (Zurich, Switzerland). A new silver technique for demonstrating terminal axon degeneration in the central nervous system.

Palumbi, G. (Pavia, Italy). A new nervous apparatus of receptive type in the cochlea of the human ear.

Quilliam, T. A. (London, G.B.). Diameter variations in nerve fibres along normal and regenerating sensory nerve trunks.

Scharrer, E. A. and Scharrer, Berta V. (Denver, U.S.A.). Neuro-secretion.

Scholl, D. A. (London, G.B.). Methods for the quantitative study of the histology of the cerebral cortex.

Sitaramayya, C. (London, G.B.). Nerve endings in the diaphragm.

Thomas, P. K. and Vizoso, A. D. (London, G.B.). The internodal length of peripheral nerve fibres.

Vonwiller, P. (Rheinau, Switzerland). New colorations of the nervous system.

Warwick, R. (Manchester, G.B.). The topography of the oculomotor nuclei of the macaque monkey.

Weber, J. A. (Geneva, Switzerland). Altérations physiologiques des terminaisons nerveuses aux différents âges, chez les Mammifères.

Zander, E. and Weddell, G. (Oxford, G.B.). The innervation of the cornea.

Zorzoli, G. and Maggi, G. (Pavia, Italy). A contribution to the knowledge of the spinal sympathetic system.

Histology

Ashford, C. A., Robinson, F. B., and Yoffey, J. M. (Bristol, G.B.). The Schultz reaction in the 'loaded' suprarenal of the adult male rat after injections of adrenaline tartrate, noradrenaline, and exposure to cold.

Barer, Gwenda R. (Oxford, G.B.). Radiography of lymphatics.

Baxter, J. S. (Bristol, G.B.). Phosphatases and Feulgen reaction in developing renal tubules.

Baxter, J. S. (Bristol, G.B.). Alkaline phosphatase in primordial germ cells and gonads in man.

BORGHESE, E., PERRI, G. C. and GELMETTI, L. (Pavia, Italy). The culture *in vitro* of embryonic megaloblasts (primitive erythroblasts).

FAYER, A. (S. Paulo, Brazil). L'existence de phosphatase acide dans les cellules cultivées *in vitro*.

FAYER, A. (S. Paulo, Brazil). Processus de clasmatose dans les cellules cultivées *in vitro*.

FOX, C. A., UBEDA-PURKISS, M., IHRIG, H. K. and BIAGIOLI, D. (Milwaukee, U.S.A.). A zinc chromate modification of the Golgi technique.

KING, T. S. (Sheffield, G.B.). Nerve cells in the ventricles of the hearts of mammals.

KLEISS, E. (Munich, Germany). An injection method for the demonstration of the vascular system.

de KOCK, L. (Aberdeen, G.B.). Carotid body structures.

LEACH, E. H. (Oxford, G.B.). Technique for staining thick sections.

LEACH, E. H. and WILLMER, E. N. (Oxford, G.B.). The structure of the retina.

LEVI, G. (Turin, Italy). Nombre et volume des neurones dans deux espèces de Caviides de taille différente.

LEVI, G. (Turin, Italy) and FAYER, A. (S. Paulo, Brazil). Distribution de la phosphatase alcaline dans les cellules cultivées *in vitro*.

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REVIEWS

External Morphology of the Primate Brain. By CORNELIUS J. CONNOLLY. (Pp. 378; 337 figs.; 72*s.* net.) Illinois: Charles C. Thomas; and Oxford, Blackwell Scientific Publications. (1949).

The scope of this book is even more restricted than its title implies, for it is concerned almost entirely with the form and fissuration of the cerebral hemispheres of the Primates. A short account is given of the sulcal pattern in the lower Primates (with the curious omission of the aberrant *Daubentonia*). A considerable section is devoted to a detailed study of the cerebral cortex in Whites and Negroes. No very clear distinction is found between these two groups, and the small differences which are observed are not related by the author to any supposed functional differences. A chapter on endocranial casts deals only with those of anthropoid apes and man. While drawing attention to certain positive facts which the evidence of such casts can provide on the sulcal pattern, the author agrees with the now generally accepted conclusion that this evidence is, in fact, usually very meagre. Thus, inferences based on a study of endocranial casts of fossil human skulls need to be very carefully scrutinized. In a final chapter, the morphological basis of sulcal formation is discussed, and it is suggested that 'homologous' sulci have a genetic basis and develop independently of mechanical factors, and that they may even be independent of the differential growth of cortical areas. This conception, which seems to involve the idea that a sulcus is a sort of theoretical abstraction, is not easy to follow. The book is excellently illustrated, and the photographs and diagrams of the sulcal pattern in Primate brains provide a useful reference for the study of individual and specific variations.

W. E. LE GROS CLARK

A History of the Teaching of Anatomy in Oxford. By H. M. SINCLAIR and A. H. T. ROBB-SMITH. (1950. Pp. 81; illustrated with frontispiece, title-page and 21 plates of 39 illustrations; 8vo; paper, 3*s.* 6*d.* net.) Oxford University Press.

As Bodley's Librarian explains in his Foreword, this booklet originated in connexion with the Exhibition illustrating the History of the Teaching of Anatomy in Oxford held, from February to July 1950, in the Radcliffe Science Library and arranged under the direction of the Superintendent of that Library. It was a happy coincidence that the International Anatomical Conference of 1950 was held in Oxford in July, and it was a nice thought that prompted the organizers of the Congress to present every member of the Congress with a copy of this booklet.

Though the brochure does contain, as its fourth appendix, a catalogue of the Exhibits in the Radcliffe Science Library, it is much more than a guide to the Exhibition and will be of lasting interest and value to all who care for the history of Anatomy, and it will be appreciated particularly by those who have been fortunate enough to visit the Colleges mentioned and to see the originals of the excellent illustrations which adorn the book. Though it is a short book, of less than one hundred pages, much time and care must have been devoted to its preparation, and the authors and publishers deserve great praise for the result, and for the very moderate price at which it is issued. The book is written in a pleasant and very readable style, with touches of humour here and there so that one can often almost see the people described. They are made yet more life-like by the beautiful reproductions of portraits that formed part of the Exhibition.

The book does not contain an index, but, what is almost better, the illustrations have below them the number of the page on which they are chiefly discussed.

Many members of the International Anatomical Congress must have been interested to know the history of the title of the President of the Congress as Dr Lee's Professor of

Anatomy. There is a romance about these titles, Dr Lee's Professor, Tomlins Reader, Aldrichian Praelector, which must be a great pleasure to their holders. The history and much of the romance of these titles is given by Dr Sinclair and Dr Robb-Smith.

An interesting appendix is the first, which consists of a 'Chronological Table of Anatomical Teachers' in Oxford from 1612 to the present day. It contains such great names as Willis, Lower, Robert Boyle, and others less well known; but nearly all will have some special interest for one reader or another. In the text, too, are many famous names, the Hunters, Richard Owen, Darwin, Pusey, Barclay, Ray Lankester, which, though not all strictly anatomical, form links in the great chain of scientific progress that such a book as this unfolds.

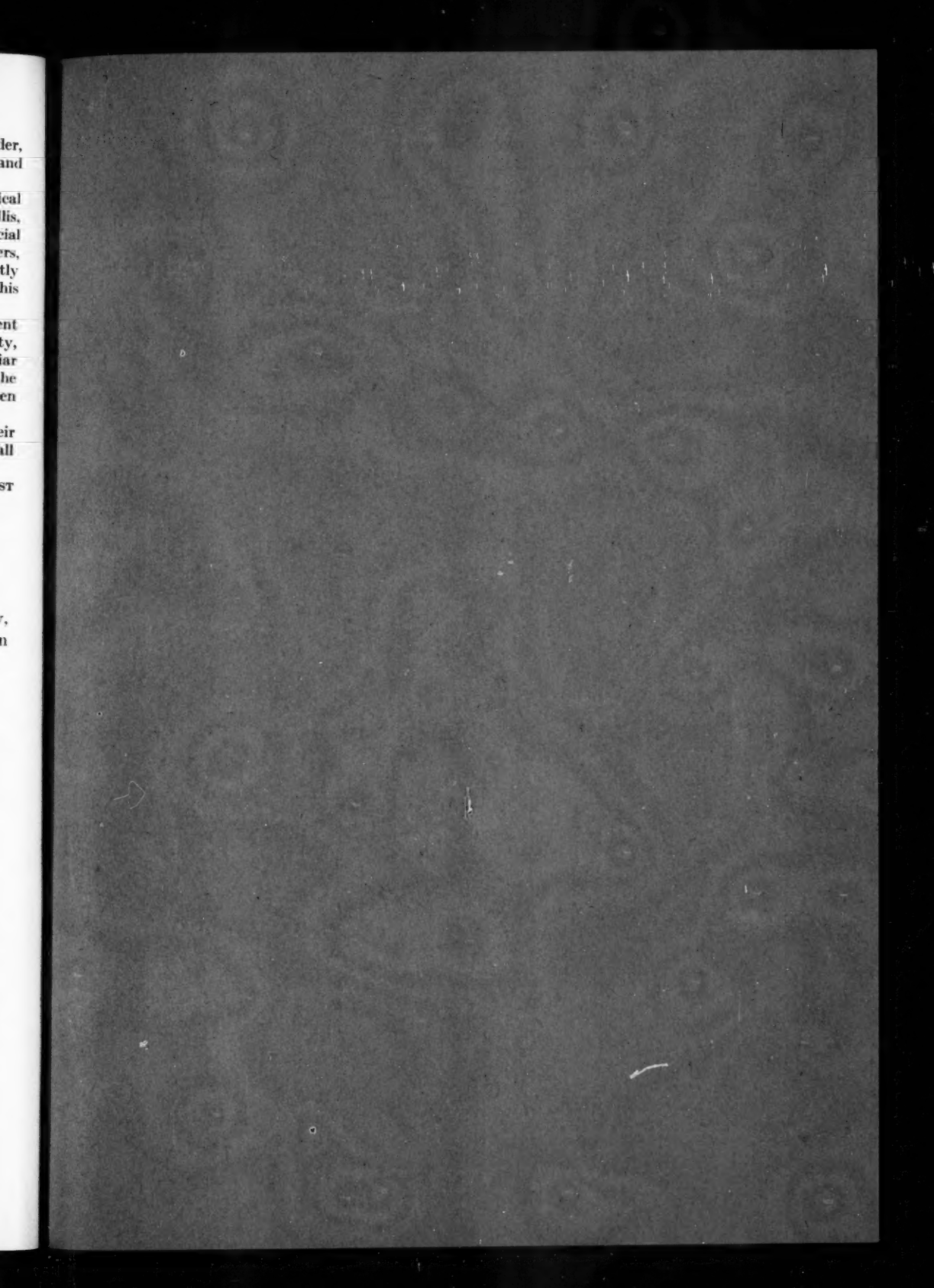
Dr Lee's Professor of Anatomy in his Preface to the book suggests that if the student 'can be presented with the story of the development of his subject in his *own* University, with records of personalities and events which have immediate reference to the familiar environment in which he himself is actually working, he is likely to be imbued with the inspiration of history in a way which would not otherwise be possible' and that he may then of his own accord read more widely.

Dr Sinclair and Dr Robb-Smith have presented a story which both students and their teachers, and not in Oxford only, may read with profit and pleasure, and for which all anatomists should give them and the publishers their thanks and praise.

C. M. WEST

OBITUARY NOTE

We have recently heard with much regret of the death of Professor A. Low, Dr G. D. Channell and Professor L. R. Shore. Obituary notices will appear in our next issue.



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9. Fifty gratis copies are given to each contributor.

10. The Journal is issued at the price of £3. 3*s.* 0*d.* per volume. Subscriptions, payable in advance, should be sent to the Cambridge University Press, Bentley House, 200 Euston Road, London, N.W. 1. Enquiries from the U.S.A. should be addressed to the Cambridge University Press American Branch, 51 Madison Avenue, New York, 10.

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